PB83-201798

Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater

(U.S.) Environmental Monitoring and Support Lab.
Cincinnati, OH

Jul 82

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA 600/4-82-057

2.

3. R
PB83-201798

4. TITLE AND SUBTITLE
Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater

5. REPORT DATE
July 1982

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
James E. Longbottom and James J. Lichtenberg, Editors

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Organic Analyses Section
Physical and Chemical Methods Branch
Environmental Monitoring and Support Laboratory

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Environmental Monitoring and Support Laboratory
Office of Research & Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

13. TYPE OF REPORT AND PERIOD COVERED
Manual

14. SPONSORING AGENCY CODE
EPA 600/06

15. SUPPLEMENTARY NOTES

16. ABSTRACT

To provide the Effluent Guidelines Division of USEPA with a means of measuring the concentration of pollutants in municipal and industrial wastewaters, the USEPA's Environmental Monitoring and Support Laboratory in Cincinnati, and the Environmental Research Laboratory in Athens, Georgia, collaborated on a research project that resulted in an analytical protocol that was successfully applied to a variety of wastewaters. The gas chromatography/mass spectrometry (GC/MS) procedures in that protocol for the measurement of organic pollutants were the forerunners of methods 624 and 625 that appear in this manual.

In a parallel research project, the Environmental Monitoring and Support Laboratory in Cincinnati, through a series of research contract and in-house activities, under- a systematic study of the analytical behavior or each of the individual classes of organic compounds to identify or develop non-MS approaches that could be used for routine monitoring of regulated discharges. This approach was pursued to minimize the requirements for expensive mass spectrometer equipment and the skilled operators required to use it. The resulting test procedures were identified as methods 601 through 612 and were first published in early 1979, along with a GC/MS method for the measurement of TCDD.

All fifteen of the test procedures were proposed as amendments to the "Guidelines Establishing Test Procedures for the Analyses of Pollutants" (40 CFR, Part 136) in December 3, 1979 . These guidelines are required by Section 304(h) of the Clean Water Act.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT Release to Public	**19. SECURITY CLASS** *(This Report)* Unclassified	**21. NO. OF PAGES** 162
	20. SECURITY CLASS *(This page)* Unclassified	**22. PRICE**

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

1<

Foreword

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory—Cincinnati, conducts research to:

- Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

Under authority of Sections 304(h) and 501(a) of the Federal Water Pollution Control Act of 1972 and the Clean Water Act of 1977, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The test procedures in this manual have undergone extensive laboratory testing and public review. They represent the state-of-the-art for the measurement of specific organic analytes in municipal and industrial wastewater.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and Support Laboratory

2<

Acknowledgments

Throughout the development and refinement of the methods for organic priority pollutants, the staff of the Physical and Chemical Methods Branch of the Environmental Monitoring and Support Laboratory—Cincinnati, has operated as a working committee to test, validate, and edit these methods. The contributions of the committee members are gratefully acknowledged:

Thomas A. Bellar
Dr. Stephen Billets
Dr. William L. Budde
Dr. Denis L. Foerst
Dr. John A. Glaser
Dr. Fred K. Kawahara
Edward H. Kerns

The outstanding efforts of typing, proofreading, and computerized text editing, performed under a series of critical deadlines by Jean Wilson, Carol Brockhoff, Beth Casias, and Patricia Hurr, are also gratefully acknowledged.

3<

Contents

Foreword iii
Acknowledgments iv
Introduction vi
Approved Test Procedures for Organic Chemicals ix

- Purgeable Halocarbons .... Method 601
- Purgeable Aromatics .... Method 602
- Acrolein and Acrylonitrile .... Method 603
- Phenols .... Method 604
- Benzidines .... Method 605
- Phthalate Esters .... Method 606
- Nitrosamines .... Method 607
- Pesticides and PCBs .... Method 608
- Nitroaromatics and Isophorone .... Method 609
- Polynuclear Aromatic Hydrocarbons .... Method 610
- Haloethers .... Method 611
- Chlorinated Hydrocarbons .... Method 612
- 2,3,7,8-Tetrachlorodibenzo-p-dioxin .... Method 613
- Purgeables .... Method 624
- Base/Neutrals, Acids, and Pesticides .... Method 625

Appendix A A-1

4<

Introduction

In 1976, the U.S. Environmental Protection Agency entered into a Settlement Agreement requiring it to study and, if necessary, regulate 65 "priority" pollutants and classes of pollutants (1). This list was later defined to include 129 specific "priority pollutants." In December 1977, Congress passed the Clean Water Act of 1977 (PL 95-217) declaring the 65 pollutants and classes of pollutants to be "toxic" under Section 307(a) of the Act.

The Settlement Agreement included a rigid time schedule for the completion of industrial wastewater analytical surveys and promulgation of effluent guidelines. To provide the Effluent Guidelines Division of USEPA with a means of measuring the concentration of pollutants in these wastewaters, the USEPA's Environmental Monitoring and Support Laboratory in Cincinnati, and the Environmental Research Laboratory in Athens, Georgia, collaborated on a research project that resulted in an analytical protocol (2) that was successfully applied to a variety of wastewaters. The gas chromatography/mass spectrometry (GC/MS) procedures in that protocol for the measurement of organic pollutants were the forerunners of methods 624 and 625 that appear in this manual.

In a parallel research project, the Environmental Monitoring and Support Laboratory in Cincinnati, through a series of research contract and in-house activities, undertook a systematic study of the analytical behavior of each of the individual classes of organic compounds to identify or develop non-MS approaches that could be used for routine monitoring of regulated discharges. This approach was pursued to minimize the requirements for expensive mass spectrometer equipment and the skilled operators required to use it. The resulting test procedures were identified as methods 601 through 612 and were first published in early 1979, along with a GC/MS method for the measurement of TCDD (3,4).

All fifteen of the test procedures discussed above were proposed as amendments to the "Guidelines Establishing Test Procedures for the Analyses of Pollutants" (40 CFR, Part 136) in December 3, 1979 (5). These guidelines are required by Section 304(h) of the Clean Water Act. As a result of public comment from over 200 respondents, extensive editorial revisions were made to the methods. The majority of the revisions were made either for clarification or to add additional flexibility for the analyst. These revised methods constitute the body of this methods manual.

The methods contained herein represent an effort to provide procedures that are as uniform and cost-effective as practical for a wide cross-section of chemical compound classes. Due to the variable chemical and physical properties of the parameters under study, some compromises were made. Therefore, in some of the methods, the extraction procedures, cleanup procedures, and determinative steps are not optimum for all parameters.

A distribution list will be established for this manual. The list will be prepared from the distribution record cards that accompany this package. Future revisions of existing methods and the addition of new methods will only be sent to individuals who return these record cards. Correspondence on these methods is invited.

References

1. Natural Resources Defense Council, Inc., et al., v. Train, 8 ERC 2120 (D.D.C. 1976), modified 12 ERC 1883 (D.D.C. 1979).
2. "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants," March 1977 (revised, April 1977). U.S. Environmental Protection Agency, Effluent Guidelines Division, Washington, D.C. 20460.
3. "Methods for Organic Compounds in Municipal and Industrial Wastewater," March 1979, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.
4. "Methods for Organic Compounds in Municipal and Industrial Wastewater," April 1979, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.
5. Guidelines Establishing Test Procedures for the Analysis of Pollutants; Proposed Regulations, 40 Code of Federal Regulations (CFR), Part 136, Published in *Federal Register, 44,* 69464.

5<

Approved Test Procedures for Organic Chemicals

Section 136.3(a) of 40 Code of Federal Regulations cites approved test procedures through the use of five subtables:

Table 1A - Biological parameters
Table 1B - Inorganic and physical parameters
Table 1C - Organic chemical parameters
Table 1D - Pesticide parameters
Table 1E - Radiological parameters

The test procedures in this manual are cited in Tables 1C and 1D. These tables have been reproduced here for the convenience of the user.

6<

Table 1C. *List of approved test procedures for organic compounds*

Parameter[1]	GC	EPA Method Number[2] GC/MS	HPLC	Other
1. Acenaphthene	610	625	610	- -
2. Acenaphthalene	610	625	610	- -
3. Acrolein	603	624[4]	- -	- -
4. Acrylonitrile	603	624[4]	- -	- -
5. Anthracene	610	625	610	- -
6. Benzene	602	624	- -	- -
7. Benzidine	- -	625[5]	605	Note 3, p. 1; Note 6, p. S48
8. Benzo(a)anthracene	610	625	610	- -
9. Benzo(a)pyrene	610	625	610	- -
10. Benzo(b)fluoranthene	610	625	610	- -
11. Benzo(ghi)perylene	610	625	610	- -
12. Benzo(k)fluoranthene	610	625	610	- -
13. Benzyl Chloride	- -	- -	- -	Note 3, p. 130; Note 6, p.S102
14. Benzyl Butyl Phthalate	606	625	- -	- -
15. Bis(2-chloroethyl) ether	611	625	- -	- -
16. Bis(2-chloroethoxy) methane	611	625	- -	- -
17. Bis(2-chloroisopropyl) ether	611	625	- -	- -
18. Bis(2-ethylhexyl) phthalate	606	625	- -	- -
19. Bromodichloromethane	601	624	- -	- -
20. Bromoform	601	624	- -	- -
21. Bromoethane	601	624	- -	- -
22. 4-Bromophenylphenyl ether	611	625	- -	- -
23. Carbon tetrachloride	601	624	- -	Note 3, p. 130; Note 6, p.S102
24. 4-Chloro-3-methylphenol	604	625	- -	- -
25. Chlorobenzene	601,602	624	- -	Note 3, p.130; Note 6, p. S102
26. Chloroethane	601	624	- -	- -
27. 2-Chloroethylvinyl ether	601	624	- -	- -
28. Chloroform	601	624	- -	Note 3, p. 130; Note 6, p.S102
29. Chloromethane	601	624	- -	- -
30. 2-Chloronaphthalene	612	625	- -	- -
31. 2-Chlorophenol	604	625	- -	- -
32. 4-Chlorophenylphenyl ether	611	625	- -	- -
33. Chrysene	610	625	610	- -
34. Dibenzo(a,h)anthracene	610	625	610	- -
35. Dibromochloromethane	601	624	- -	- -
36. 1,2-Dichlorobenzene	601, 602, 612	624, 625	- -	- -
37. 1,3-Dichlorobenzene	601, 602, 612	624, 625	- -	- -
38. 1,4-Dichlorobenzene	601, 601, 612	624, 625	- -	- -
39. 3,3'-Dichlorobenzidine	- -	625	605	- -
40. Dichlorodifluoromethane	601	- -	- -	- -
41. 1,1-Dichloroethane	601	624	- -	- -
42. 1,2-Dichloroethane	601	624	- -	- -
43. Dichloroethene	601	624	- -	- -
44. trans-1,2-Dichloroethene	601	624	- -	- -
45. 2,4-Dichlorophenol	604	625	- -	- -
46. 1,2-Dichloropropane	601	624	- -	
47. cis-1,3-Dichloropropene	601	624	- -	- -
48. trans-1,3-Dichoropropene	601	624	- -	- -
49. Diethyl phthalate	606	625	- -	- -
50. 2,4-Dimethylphenol	604	625	- -	- -
51. Dimethyl phthalate	606	625	- -	- -
52. Di-n-butyl phthalate	606	625	- -	- -
53. Di-n-octyl phthalate	606	625	- -	- -
54. 2,4-Dinitrophenol	604	625	- -	- -
55. 2,4-Dinitrotoluene	609	625	- -	- -
56. 2,6-Dinitrotoluene	609	625	- -	- -

7<

Table 1C. *Continued*

	EPA Method Number[2]			
Parameter[1]	GC	GC/MS	HPLC	Other
57. Epichlorohydrin	- -	- -	- -	Note 3, p.130; Note 6, p.S102
58. Ethylbenzene	602	624	- -	- -
59. Fluoranthene	610	625	610	- -
60. Fluorene	610	625	610	- -
61. Hexachlorobenzene	612	625	- -	- -
62. Hexachlorobutadiene	612	625	- -	- -
63. Hexachlorocyclopentadiene	612	625[5]	- -	- -
64. Hexachloroethane	612	625	- -	- -
65. Ideno(1,2,3-cd)pyrene	610	625	610	- -
66. Isophorone	609	625	- -	- -
67. Methylene chloride	601	624	- -	Note 3, p. 130; Note 6, p.S102
68. 2-Methyl-4,6-dinitrophenol	604	625	- -	- -
69. Naphthalene	610	625	- -	- -
70. Nitrobenzene	609	625	- -	- -
71. 2-Nitrophenol	604	625	- -	- -
72. 4-Nitrophenol	604	625	- -	- -
73. N-Nitrosodimethylamine	607	625[5]	- -	- -
74. N-Nitrosodi-n-propylamine	607	625	- -	- -
75. N-Nitrosodiphenylamine	607	625[5]	- -	- -
76. PCB-1016	608	625	- -	Note 3, p. 43; Note 6, p. S78
77. PCB-1221	608	625	- -	Note 3, p. 43; Note 6, p. S78
78. PCB-1232	608	625	- -	Note 3, p. 43; Note 6, p.S78
79. PCB-1242	608	625	- -	Note 3, p. 43; Note 6, p. S78
80. PCB-1248	608	625	- -	Note 3, p. 43; Note 6, p. S78
81. PCB-1254	608	625	- -	Note 3, p.43; Note 6, p. S78
82. PCB-1260	608	625	- -	Note 3, p. 43; Note 6, p. S78
83. Pentachlorophenol	604	625	- -	Note 3, p. 140; Note 6, p. S50
84. Phenanthrene	610	625	610	- -
85. Phenol	604	625	- -	- -
86. Pyrene	610	625	610	- -
87. 2,3,7,8-Tetrachloro-dibenzo-p-dioxin	- -	613	- -	- -
88. 1,1,2,2-Tetrachloroethane	601	624	- -	Note 3, p. 130; Note 6, p. S102
89. Tetrachloroethene	601	624	- -	Note 3, p. 130 Note 6, p. S102
90. Toluene	602	624	- -	- -
91. 1,2,4-Trichlorobenzene	612	625	- -	Note 3, p. 130; Note 6, p. S102
92. 1,1,1-Trichloroethane	601	624	- -	- -
93. 1,1,2-Trichloroethane	601	624	- -	Note 3, p. 130; Note 6, p.S102
94. Trichloroethene	601	624	- -	- -
95. Trichlorofluoromethane	601	624	- -	- -
96. 2,4,6-Trichlorophenol	604	625	- -	- -
97. Vinyl Chloride	601	624	- -	- -

8<

Table 1C. *Notes*

[1] All parameter concentrations are expressed in micrograms per liter (µg/L).

[2] "Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater," USEPA, July 1982.

[3] "Methods for Benzidine, Chlorinated Organic Compounds, Pentachlorophenol and Pesticides in Water and Wastewater," USEPA, September 1978.

[4] Method 624 may be extended to screen samples for acrolein and acrylonitrile. However, when they are known to be present, the preferred method for these two compounds is Method 603.

[5] Method 625 may be extended to include benzidine, hexachlorocyclopentadiene, N-nitrosodimethylamine, and N-nitrosodiphenylamine. However, when they are known to be present, Method 605, 612, 607, and 607, respectively, are the preferred methods for these compound.

[6] "Selected Analytical Methods Approved and Cited by the United States Environmental Protection Agency," Supplement to the Fifteenth Edition of Standard Methods for the Examination of Water and Wastewater (1981).

40 C.F.R. Part 136, § 136.3

Table 1D. *List of approved test procedures for pesticides.*[1]

Parameter (µg/L)	Method	EPA[2]	Std. Methods 15th Ed.	ASTM	Other
1. Aldrin	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30
	GC/MS	625	----	----	
2. Ametryn	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
3. Aminocarb	TLC	---	----	----	Note 3, p. 94; Note 6, p. S16
4. Atraton	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
5. Atrazine	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
6. Azinphos methyl	gc	---	----	---- ----	Note 3, p. 25; Note 6, p.S51
7. Barban	TLC	---	----	----	znote 3, p. 104; Note 6, p. S64
8. α-BHC	GC	608	509A	D3086	Note 3, p. 7
	GC/MS	625[5]	----	----	----
9. β-BHC	GC	608	----	D3086	---
	GC/MS	625	---	----	----
10. δ-BHC	GC	608	----	D3086	----
	GC/MS	625[5]	----	----	----
11. δ-BHC (Lindane)	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30, Note 6, p. S73
	GC/MS	625	----	----	----
12. Captan	GC	---	509A	----	Note 3, p. 7
13. Carbaryl	TLC	---	----	----	Note 3, p. 94; Note 6, p. S73
14. Carbophenothion	GC	---	----	----	Note 4, p. 30; Note 6, p. S73
15. Chlordane	GC	608	509A	D3086	Note 3, p. 7
	GC/MS	625	----	----	----
16. Chlorpropham	TLC	---	----	----	Note 3, p. 104; Note 6, p.S64
17. 2,4-D	GC	---	509B	----	Note 3, p. 115; Note 4, p. 35
18. 4,4'-DDD	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30
	GC/MS	625	----	----	----
19. 4,4'-DDE	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30
	GC/MS	625	----	----	----
20. 4,4'-DDT	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30
	GC/MS	625	----	----	----
21. Demeton-O	GC	---	----	----	Note 3, p. 25; Note 6, p. S51

Table 1D. *Continued*

Parameter (μg/L)	Method	EPA[2]	Std. Methods 15th Ed.	ASTM	Other
22. Demeton-S	GC	---	----	----	Note 3, p. 25; Note 6, p. S51
23. Diazinon	GC	---	----	----	Note 3, p. 25; Note 4, p. 30; Note 6, p. S51
24. Dicamba	GC	---	----	----	Note 3, p. 115
25. Dichlofenthion	GC	---	----	----	Note 4, p. 30; Note 6, p. S73
26. Dichloran	GC	---	509A	----	Note 3, p. 7
27. Dicofol	GC	---	----	D3086	----
28. Dieldrin	GC	608	509A	----	Note 3, p. 7; Note 4, p. 30 Note 6, p. S73
	GC/MS	625	----	----	----
29. Dioxathion	GC	---	----	----	Note 4, p. 30; Note 6, p. S73
30. Disulfoton	GC	---	----	----	Note 3, p. 25; Note 6, p. S51
31. Diuron	TLC	---	----	----	Note 3, p. 104; Note 6, p.S64
32. Endosulfan I	GC	608	509A	D3086	Note 3, p. 7
	GC/MS	625[5]	----	----	----
33. Endosulfan II	GC	608	509A	D3086	Note 3, p. 7
	GC/MS	625[5]	----	----	----
34. Endosulfan sulfate	GC	608	----	----	----
	GC/MS	625	----	----	----
35. Endrin	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30
	GC/MS	625[5]	----	----	----
36. Endrin aldehyde	GC	608	----	----	----
	GC/MS	625	----	----	----
37. Ethion	GC	---	----	----	Note 4, p. 30; Note 6, p. S64
38. Fenuron	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
39. Fenuron-TCA	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
40. Heptachlor	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 40
	GC/MS	625	----	----	----
41. Heptachlor epoxide	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30; Note 6, p. S73
	GC/MS	625	----	----	----
42. Isodrin	GC	---	----	----	Note 4, p. 30; Note 6, p. S73
43. Linuron	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
44. Malathion	GC	---	509A	----	Note 3, p. 25; Note 4, p. 30; Note 6, p. S51
45. Methiocarb	TLC	---	----	----	Note 3, p. 94; Note 6, p. S60
46. Methoxychlor	GC	---	509A	D3086	Note 3, p. 7; Note 4, p. 30
47. Mexacarbate	TLC	---	----	----	Note 3, p. 94; Note 6, p. S60
48. Mirex	GC	---	509A	----	Note 3, p. 7
49. Monuron	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
40. Monuron-TCA	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64

10<

Table 1D. *Continued*

Parameter (µg/L)	Method	EPA[2]	Std. Methods 15th Ed.	ASTM	Other
51. Neburon	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
52. Parathion methyl	GC	---	509A	----	Note 3, p. 25; Note 4, p. 30
53. Parathion ethyl	GC	---	509A	----	Note 3, p. 25
54. PCNB	GC	---	509A	----	Note 3, p. 7
55. Perthane	GC	---	----	D3086	----
56. Prometon	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
57. Prometryn	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
58. Propazine	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
59. Propham	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
60. Propoxur	TLC	---	----	----	Note 3, p. 94; Note 6, p. S60
61. Secbumeton	TLC	---	----	----	Note 3, p. 83; Note 6, p. S68
62. Siduron	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
63. Simazine	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
64. Strobane	GC	---	509A	----	Note 3, p. 7
65. Swep	TLC	---	----	----	Note 3, p. 104; Note 6, p. S64
66. 2,4,5-T	GC	---	509B	----	Note 3, p. 115; Note 4, p. 35
67. 2,4,5-tp (Silvex)	GC	---	509B	----	Note 3, p. 83; Note 6, p. S68
68. Terbuthylazine	GC	---	----	----	Note 3, p. 83; Note 6, p. S68
69. Toxaphene	GC	608	509A	D3086	Note 3, p. 7; Note 4, p. 30
	GC/MS	625	----	----	----
70. Trifluralin	GC	---	----	----	Note 3, p. 7

---

[1] Pesticides are listed in this table by common name for the convenience of the reader. Additional pesticides may be found under Table 1C, where entries are listed by chemical name.

[2] "Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater." USEPA, July 1982.

[3] "Methods for Benzidine, Chlorinated Organic Compounds, Pentachlorophenol and Pesticides in Water and Wastewater," USEPA, September 1978.

[4] Methods for Analysis of Organic Substances in Water, U.S. Geological Survey Techniques of Water Resources Inv., Book 5, Ch. A3 (1972), p. 30.

[5] The method may be extended to screen samples for α-BHC, and δ-BHC, endosulfan I, endosulfan II, and endrin. However, when they are known to be present, the referenced gas chromatographic procedures are the preferred methods.

[6] "Selected Analytical Methods Approved and Cited by the United States Environmental Protection Agency," Supplement to the Fifteenth Edition of Standard Methods for the Examination of Water and Wastewater (1981).

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Purgeable Halocarbons—Method 601

1. Scope and Application

1.1 This method covers the determination of 29 purgeable halocarbons. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Bromodichloromethane	32101	75-27-4
Bromoform	32104	75-25-2
Bromomethane	34413	74-83-9
Carbon tetrachloride	32102	56-23-5
Chlorobenzene	34301	108-90-7
Chloroethane	34311	75-00-3
2-Chloroethylvinyl ether	34576	100-75-8
Chloroform	32106	67-66-3
Chloromethane	34418	74-87-3
Dibromochloromethane	32105	124-48-1
1,2-Dichlorobenzene	34536	95-50-1
1,3-Dichlorobenzene	34566	541-73-1
1,4-Dichlorobenzene	34571	106-46-7
Dichlorodifluoromethane	34668	75-71-8
1,1-Dichloroethane	34496	75-34-3
1,2-Dichloroethane	34531	107-06-2
1,1-Dichloroethene	34501	75-35-4
trans-1,2-Dichloroethene	34546	156-60-5
1,2-Dichloropropane	34541	78-87-5
cis-1,3-Dichloropropene	34704	10061-01-5
trans-1,3-Dichloropropene	34699	10061-02-6
Methylene chloride	34423	75-09-2
1,1,2,2-Tetrachloroethane	34516	79-34-5
Tetrachloroethene	34475	127-18-4
1,1,1-Trichloroethane	34506	71-55-6
1,1,2-Trichloroethane	34511	79-00-5
Trichloroethene	39180	79-01-6
Trichlorofluoromethane	34488	75-69-4
Vinyl chloride	39175	75-01-4

1.2 This is a purge and trap gas chromatographic method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identification should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 624 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for most of the parameters listed above.

1.3 The method detection limit (MDL, defined in Section 12.1)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the operation of a purge and trap system and a gas chromatograph and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 An inert gas is bubbled through a 5-mL water sample contained in a specially-designed purging chamber at ambient temperature. The halocarbons are efficiently transferred from the aqueous phase to the vapor phase. The vapor is swept through a sorbent trap where the halocarbons are trapped. After purging is completed, the trap is heated and backflushed with the inert gas to desorb the halocarbons onto a gas chromatographic column. The gas chromatograph is temperature programmed to separate the halocarbons which are then detected with a halide-specific detector.(2,3)

2.2 The method provides an optional gas chromatographic column that may be helpful in resolving the compounds of interest from interferences that may occur.

3. Interferences

3.1 Impurities in the purge gas and organic compounds out-gassing from the plumbing ahead of the trap account for the majority of contamination problems. The analytical system must be demonstrated to be free from contamination under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5. The use of non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purging device should be avoided.

3.2 Samples can be contaminated by diffusion of volatile organics (particularly fluorocarbons and methylene chloride) through the septum seal into the sample during shipment and storage. A field reagent blank prepared from reagent water and carried through the sampling and handling protocol can serve as a check on such contamination.

3.3 Contamination by carry-over can occur whenever high level and low level samples are sequentially analyzed. To reduce carry-over, the purging device and sample syringe must be rinsed with reagent water between sample analyses. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of reagent water to check for cross contamination. For samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds or high organohalide levels, it may be necessary to wash out the purging device with a detergent solution, rinse it with distilled water, and then dry it in a 105 °C oven between analyses. The trap and other parts of the system are also subject to contamination; therefore, frequent bakeout and purging of the entire system may be required.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(4-6) for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: carbon tetrachloride, chloroform, 1,4-dichlorobenzene, and vinyl chloride. Primary standards of these toxic compounds should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete sampling.

5.1.1 Vial—25-mL capacity or larger, equipped with a screw cap with hole in center (Pierce #13075 or equivalent). Detergent wash, rinse cap with tap and distilled water, and dry at 105 °C before use.

5.1.2 Septum—Teflon-faced silicone (Pierce #12722 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105 °C for one hour before use.

5.2 Purge and trap device—The purge and trap device consists of three separate pieces of equipment: the sample purger, trap, and the desorber. Several complete devices are now commercially available.

5.2.1 The sample purger must be designed to accept 5-mL samples with a water column at least 3 cm deep. The gaseous head space between the water column and the trap must have a total volume of less than 15-mL. The purge gas must pass through the water column as finely divided bubbles with a diameter of less than 3 mm at the origin. The purge gas must be introduced no more than 5 mm from the base of the water column. The sample purger, illustrated in Figure 1, meets these design criteria.

5.2.2 The trap must be at least 25 cm long and have an inside diameter of at least 0.105 inch. The trap must be packed to contain the following minimum lengths of adsorbents: 1.0 cm of methyl silicone coated backing (Section 6.3.3), 7.7 cm of 2,6-diphenylene oxide polymer (Section 6.3.2), 7.7 cm of silica gel, 7.7 gm of coconut charcoal (Section 6.3.1). If it is not necessary to analyze for dichlorodifluroromethane, the charcoal can be eliminated, and the polymer section lengthened to 15 cm. The minimum specifications for the trap are illustrated in Figure 2.

5.2.3 The desorber must be capable of rapidly heating the trap to 180 °C. The polymer section of the trap should not be heated higher than 180 °C and the remaining sections should not exceed 220 °C. The desorber design, illustrated in Figure 2, meets these criteria.

5.2.4 The purge and trap device may be assembled as a separate unit or be coupled to a gas chromatograph as illustrated in Figures 3 and 4.

5.3 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.3.1 Column 1—8 ft long × 0.1 in ID stainless steel or glass, packed with 1% SP-1000 on Carbopack B (60/80 mesh) or equivalent. This column was used to develop the method performance statements in Section 12. Guidelines for the use of alternate column packings are provided in Section 10.1.

5.3.2 Column 2—6 ft long × 0.1 in ID stainless steel or glass, packed with chemically bonded n-octane on Porasil-C (100/120) mesh or equivalent.

5.3.3 Detector—Electrolytic conductivity or microcoulometric. These types of detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope. The electrolytic conductivity detector was used to develop the method performance statements and MDL listed in Tables 1 and 2. Guidelines for the use of alternate detectors are provided in Section 10.1.

5.4 Syringes—5-mL glass hypodermic with Luerlok tip (two each), if applicable to the purging device.

5.5 Micro syringes—25 μL, 0.006 in ID needle.

5.6 Syringe valve—2-way, with Luer ends (three each).

5.7 Syringe—5-mL, gas-tight with shut-off valve.

5.8 Bottle—15-mL, screw cap, with Teflon cap liner.

5.9 Balance—Analytical, capable of accurately weighing 0.0001 g.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of the parameters of interest.

6.1.1 Reagent water can be generated by passing tap water through a carbon filter bed containing about 1 lb. of activated carbon (Filtrasorb-300 or equivalent (Calgon Corp.)).

6.1.2 A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

6.1.3 Reagent water may also be prepared by boiling water for 15 minutes. Subsequently, while maintaining the temperature at 90 °C, bubble a contaminant-free inert gas through the water for one hour. While still hot, transfer the water to a narrow mouth screw-cap bottle and seal with a Teflon-lined septum and cap.

6.2 Sodium thiosulfate—(ACS) Granular.

6.3 Trap Materials

6.3.1 Coconut charcoal (6/10 mesh sieved to 26 mesh), (Barnaby Chaney, CA-580-26 lot # M-2649 or equivalent).

6.3.2 2,6-Diphenylene oxide polymer—Tenax, (60/80 mesh), chromatographic grade or equivalent.

6.3.3 Methyl silicone packing—3% OV-1 on 60/80 mesh Chromosorb-W or equivalent.

6.3.4 Silica gel—35/60 mesh, Davison, grade-15 or equivalent.

6.4 Methyl Alcohol—Pesticide quality or equivalent.

6.5 Stock standard solutions—Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions. Prepare stock standard solutions in methyl alcohol using assayed liquids or gas cylinders as appropriate. Because of the toxicity of some of the organohalides, primary dilutions of these materials should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be used when the analyst handles high concentrations of such materials.

6.5.1 Place about 9.8 mL of methyl alcohol into a 10-mL ground glass stoppered volumetric flask. Allow the flask to stand, unstoppered, for about 10 minutes or until all alcohol wetted surfaces have dried. Weigh the flask to the nearest 0.1 mg.

6.5.2 Add the assayed reference material:

6.5.2.1 Liquids—Using a 100-μL syringe, immediately add two or more drops of assayed reference material to the flask, then reweigh. Be sure that the drops fall directly into the alcohol without contacting the neck of the flask.

6.5.2.2 Gases—To prepare standards for any of the six halocarbons that boil below 30 °C (bromomethane, chloroethane, chloromethane, dichlorodifluoromethane, trichlorofluoromethane, vinyl chloride), fill a 5-mL valved gas-tight syringe with the reference standard to the 5.0-mL mark. Lower the needle to 5 mm above the methyl alcohol meniscus. Slowly introduce the reference standard above the surface of the liquid (the heavy gas will rapidly dissolve into the methyl alcohol).

6.5.3 Reweigh, dilute to volume, stopper, then mix by inverting the flask several times. Calculate the concentration in micrograms per microliter from the net gain in weight. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.5.4 Transfer the stock standard solution into a Teflon-sealed screw-cap bottle. Store, with minimal headspace, at −10 to −20 °C and protect from light.

6.5.5 Prepare fresh standards weekly for the six gases and 2-chloroethylvinyl ether. All other standards must be replaced after one month, or sooner if comparison with check standards indicate a problem.

6.6 Secondary dilution standards—Using stock standard solutions, prepare secondary dilution standards in methyl alcohol that contain the compounds of interest, either singly or mixed together. The secondary dilution standards should be prepared at concentrations such that the aqueous calibration standards prepared in Sections 7.3.1 or 7.4.1 will bracket the working range of the analytical system. Secondary dilution standards should be stored with minimal headspace and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, in Cincinnati, Ohio.

7. Calibration

7.1 Assemble a purge and trap device that meets the specifications in Section 5.2. Condition the trap overnight at 180 °C by backflushing with an inert gas flow of at least 20 mL/min. Prior to use, daily condition traps 10 minutes while backflushing at 180 °C.

7.2 Connect the purge and trap device to a gas chromatograph. The gas chromatograph must be operated using temperature and flow rate parameters equivalent to those in Table 1. Calibrate the purge and trap-gas chromatographic system using either the external standard technique (Section 7.3) or the internal standard technique (Section 7.4).

7.3 External standard calibration procedure:

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter by carefully adding 20.0 µL of one or more secondary dilution standards to 100, 500, or 1000 mL of reagent water. A 25-µL syringe with a 0.006 inch ID needle should be used for this operation. One of the external standards should be at a concentration near, but above, the method detection limit (See Table 1) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector. These aqueous standards can be stored up to 24 hours, if held in sealed vials with zero headspace as described in Section 9.2. If not so stored, they must be discarded after one hour.

7.3.2 Analyze each calibration standard according to Section 10, and tabulate peak height or area responses versus the concentration in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range ($<$10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.3.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.4 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples. The compounds recommended for use as surrogate spikes in Section 8.7 have been used successfully as internal standards, because of their generally unique retention times.

7.4.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest as described in Section 7.3.1.

7.4.2 Prepare a spiking solution containing each of the internal standards using the procedures described in Sections 6.5 and 6.6. It is recommended that the secondary dilution standard be prepared at a concentration of 15 µg/mL of each internal standard compound. The addition of 10µL of this standard to 5.0 mL of sample or calibration standard would be equivalent to 30 µg/L.

7.4.3 Analyze each calibration standard, according to Section 10, adding 10 µL of internal standard spiking solution directly to the syringe (Section 10.4). Tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1 } RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard.

C_s = Concentration of the parameter to be measured.

If the RF value over the working range is a constant ($<$10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.4.4 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methyl alcohol 500 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a syringe, add 10 µL of the check sample concentrate to each of a minimum of four 5-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X-R| > 2p$, review potential problem areas and repeat the test.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3.1 Calculate upper and lower control limts for method performance:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (LCL) = R − 3s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(7) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.(7)

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 11.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Each day, the analyst must demonstrate through the analysis of reagent water, that interferences from the analytical system are under control.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

8.7 The analyst should maintain constant surveillance of both the performance of the analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and blank with surrogate halocarbons. A combination of bromochloromethane, 2-bromo-1-chloropropane, and 1,4-dichlorobutane is recommended to encompass the range of the temperature program used in this method. From stock standard solutions prepared as above, add a volume to give 7500 μg of each surrogate to 45 mL of reagent water contained in a 50-mL volumetric flask, mix and dilute to volume (15 ng/μL). If the internal standard calibration procedure is being used, the surrogate compounds may be added directly to the internal standard spiking solution (Section 7.4.2). Add 10 μL of this surrogate spiking solution directly into the 5-mL syringe with every sample and reference standard analyzed. Prepare a fresh surrogate spiking solution on a weekly basis.

9. Sample Collection, Preservation, and Handling

9.1 All samples must be iced or refrigerated from the time of collection until extraction. If the sample contains free or combined chlorine, add sodium thiosulfate preservative (10 mg/40 mL is sufficient for up to 5 ppm Cl_2) to the empty sample bottle just prior to shipping to the sampling site. USEPA methods 330.4 and 330.5 may be used for measurement of residual chlorine.(8) Field test kits are available for this purpose.

9.2 Grab samples must be collected in glass containers having a total volume of at least 25 mL. Fill the sample bottle just to overflowing in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottle so that no air bubbles are entrapped in it. If preservative has been added, shake vigorously for one minute. Maintain the hermetic seal on the sample bottle until time of analysis.

9.3 All samples must be analyzed within 14 days of collection.

10. Sample Extraction and Gas Chromatography

10.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this Table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 is shown in Figure 5. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

10.2 Calibrate the system daily as described in Section 7.

10.3 Adjust the purge gas (nitrogen or helium) flow rate to 40 mL/min. Attach the trap inlet to the purging device, and set the device to purge. Open the syringe valve located on the purging device sample introduction needle.

10.4 Allow sample to come to ambient temperature prior to introducing it to the syringe. Remove the plunger from a 5-mL syringe and attach a closed syringe valve. Open the sample bottle (or standard) and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. Since this process of taking an aliquot destroys the validity of the sample for future analysis, the analyst should fill a second syringe at this time to protect against possible loss of data. Add 10.0 μL of the surrogate spiking solution (8.7) and 10.0 μL of the internal standard spiking solution (Section 7.4.2), if applicable, through the valve bore, then close the valve.

10.5 Attach the syringe-syringe valve assembly to the syringe valve on the purging device. Open the syringe valves and inject the sample into the purging chamber.

10.6 Close both valves and purge the sample for 11.0 ± .1 minutes at ambient temperature.

10.7 After the 11-minute purge time, attach the trap to the chromatograph, adjust the device to the desorb mode, and begin to temperature program the gas chromatograph. Introduce the trapped materials to the GC column by rapidly heating the trap to 180 °C while backflushing the trap with an inert gas between 20 and 60 mL/min for four minutes. If rapid heating of the trap cannot be achieved, the gas chromatographic column must be used as a secondary trap by cooling it to 30 °C (subambient temperature, if poor peak geometry or random retention time problems persist) instead of the initial program temperature of 45 °C.

10.8 While the trap is being desorbed into the gas chromatograph, empty the purging chamber using the sample introduction syringe. Wash the chamber with two 5-mL flushes of reagent water.

10.9 After desorbing the sample for four minutes recondition the trap by returning the purge and trap device to the purge mode. Wait 15 seconds then close the syringe valve on the purging device to begin gas flow through the trap. The trap temperature should be maintained at 180 °C. After approximately seven minutes turn off the trap heater and open the syringe valve to stop the gas flow through the trap. When cool the trap is ready for the next sample.

10.10 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.11 If the response for the peak exceeds the working range of the system, prepare a dilution of the sample with reagent water from the aliquot in the second syringe and reanalyze.

11. Calculations

11.1 Determine the concentration of individual compounds in the sample.

11.1.1 If the external standard calibration procedure is used, calculate the concentration of material from the peak response using the calibration curve or calibration factor determined in Section 7.3.2.

11.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.4.3 and equation 2.

Eq. 2.

Concentration $\mu g/L = (A_s C_{is})/(A_{is})(RF)$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_s = Concentration of the internal standard.

11.2 Report results in micrograms per liter. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

11.3 For samples processed as part of a set where the spiked sample recovery falls outside of the control limits which were established according to Section 8.3, data for the affected parameters must be labeled as suspect.

12. Method Performance

12.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero.(1) The MDL concentrations listed in Table 1 were obtained using reagent water.(9) Similar results were achieved using representative wastewaters. The MDL actually achieved in a given analysis will vary depending on instrument sensitivity and matrix effects.

12.2 This method is recommended for use in the concentration range from the MDL up to 1000 × MDL. Direct aqueous injection techniques should be used to measure concentration levels above 1000 × MDL.

12.3 In a single laboratory (Monsanto Research), using reagent water and wastewaters spiked at or near background levels, the average recoveries presented in Table 2 were obtained(9). The standard deviation of the measurement in percent recovery is also included in Table 2(9).

12.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. Bellar, T.A., and Lichtenberg, J.J. *Journal American Water Works Association, 66,* 739, (1974).
3. Bellar, T.A., and Lichtenberg, J.J. "Semi-Automated Headspace Analysis of Drinking Waters and Industrial Waters for Purgeable Volatile Organic Compounds," Proceedings from Symposium on Measurement of Organic Pollutants in Water and Wastewater, American Society for Testing and Materials, STP 686, C.E. Van Hall, editor, 1978.
4. "Carcinogens—Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
5. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory—Cincinnati, Ohio 45268, March 1979.
8. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, U.S. Environmental Protection Agency,

Environmental Monitoring and Support Laboratory—Cincinnati, Ohio 45268, March 1979.

9. "EPA Method Validation Study 23, Method 601 (Purgeable Halocarbons)," Report for EPA Contract 68-03-2856 (In preparation).

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	Retention Time (min.) Column 1	Retention Time (min.) Column 2	Method Detection Limit µg/L
Chloromethane	1.50	5.28	0.08
Bromomethane	2.17	7.05	1.18
Dichlorodifluoromethane	2.62	nd	1.81
Vinyl chloride	2.67	5.28	0.18
Chloroethane	3.33	8.68	0.52
Methylene chloride	5.25	10.1	0.25
Trichlorofluoromethane	7.18	nd	nd
1,1-Dichloroethene	7.93	7.72	0.13
1,1-Dichloroethane	9.30	12.6	0.07
trans-1,2-Dichloroethene	10.1	9.38	0.10
Chloroform	10.7	12.1	0.05
1,2-Dichloroethane	11.4	15.4	0.03
1,1,1-Trichloroethane	12.6	13.1	0.03
Carbon tetrachloride	13.0	14.4	0.12
Bromodichloromethane	13.7	14.6	0.10
1,2-Dichloropropane	14.9	16.6	0.04
trans-1,3-Dichloropropene	15.2	16.6	0.34
Trichloroethene	15.8	13.1	0.12
Dibromochloromethane	16.5	16.6	0.09
1,1,2-Trichloroethane	16.5	18.1	0.02
cis-1,3-Dichloropropene	16.5	18.0	0.20
2-Chloroethylvinyl ether	18.0	nd	0.13
Bromoform	19.2	19.2	0.20
1,1,2,2-Tetrachloroethane	21.6	nd	0.03
Tetrachloroethene	21.7	15.0	0.03
Chlorobenzene	24.2	18.8	0.25
1,3-Dichlorobenzene	34.0	22.4	0.32
1,2-Dichlorobenzene	34.9	23.5	0.15
1,4-Dichlorobenzene	35.4	22.3	0.24

nd = not determined

Column 1 conditions: Carbopack B 60/80 mesh coated with 1% SP-1000 packed in an 8 ft × 0.1 in ID stainless steel or glass column with helium carrier gas at 40 mL/min flow rate. Column temperature held at 45°C for 3 min. then programmed at 8°C/min. to 220° and held for 15 min.

Column 2 conditions: Porasil-C 100/120 mesh coated with n-octane packed in a 6 ft × 0.1 in ID stainless steel or glass column with helium carrier gas at 40 mL/min flow rate. Column temperature held at 50°C for 3 min then programmed at 6°C/min to 170° and held for 4 min.

Table 2. *Single Operator Accuracy and Precision*

Parameter	*Average Percent Recovery*	*Standard Deviation %*	*Spike Range (μg/L)*	*Number of Analyses*	*Matrix Types*
Bromodichloromethane	*100.9*	*5.0*	*0.43-46.7*	*21*	*3*
Bromoform	*89.5*	*9.0*	*1.45-50*	*20*	*3*
Bromomethane	*105.0*	*17.3*	*3.39-49.2*	*21*	*3*
Carbon tetrachloride	*82.5*	*25.6*	*0.55-50*	*19*	*3*
Chlorobenzene	*93.9*	*8.9*	*2.21-50*	*20*	*3*
Chloroethane	*91.5*	*22.4*	*3.95-50*	*21*	*3*
2-Chloroethylvinyl ether	*96.3*	*9.9*	*4.39-133*	*20*	*3*
Chloroform	*101.7*	*20.6*	*0.44-50*	*20*	*3*
Chloromethane	*91.4*	*13.4*	*0.55-23.9*	*21*	*3*
Dibromochloromethane	*98.3*	*6.5*	*0.75-93.0*	*21*	*3*
1,2-Dichlorobenzene	*10.20*	*2.0*	*4.89-154*	*21*	*3*
1,3-Dichlorobenzene	*91.6*	*4.3*	*2.94-46.7*	*21*	*3*
1,4-Dichlorobenzene	*97.5*	*9.3*	*2.99-51.6*	*21*	*3*
Dichlorodifluoromethane	*87.8*	*18.0*	*2.18-43.4*	*21*	*3*
1,1-Dichloroethane	*102.3*	*5.5*	*0.44-46.7*	*21*	*3*
1,2-Dichloroethane	*97.8*	*4.8*	*0.44-46.7*	*21*	*3*
1,1-Dichloroethene	*101.1*	*21.7*	*0.37-50*	*19*	*3*
trans-1,2-Dichloroethene	*91.0*	*19.3*	*0.44-98.0*	*20*	*3*
1,2-Dichloropropane	*97.7*	*8.8*	*0.29-39.0*	*21*	*3*
cis-1,3-Dichloropropene	*86.7*	*6.0*	*0.44-46.7*	*21*	*3*
trans-1,3-Dichloropropene	*73.5*	*17.2*	*0.43-50*	*20*	*3*
Methylene chloride	*97.9*	*2.6*	*0.73-46.7*	*21*	*3*
1,1,2,2-Tetrachloroethane	*91.9*	*15.0*	*0.46-46.7*	*21*	*3*
Tetrachloroethene	*94.1*	*18.1*	*0.50-35.0*	*21*	*3*
1,1,1-Trichloroethane	*75.1*	*12.5*	*0.37-29.0*	*21*	*3*
1,1,2-Trichloroethane	*91.0*	*25.1*	*0.45-50*	*21*	*3*
Trichloroethene	*106.1*	*7.4*	*0.38-46.7*	*21*	*3*
Trichlorofluoromethane	*89.3*	*13.9*	*149*	*14*	*2*
Vinyl chloride	*101.9*	*11.4*	*0.82-32.3*	*21*	*3*

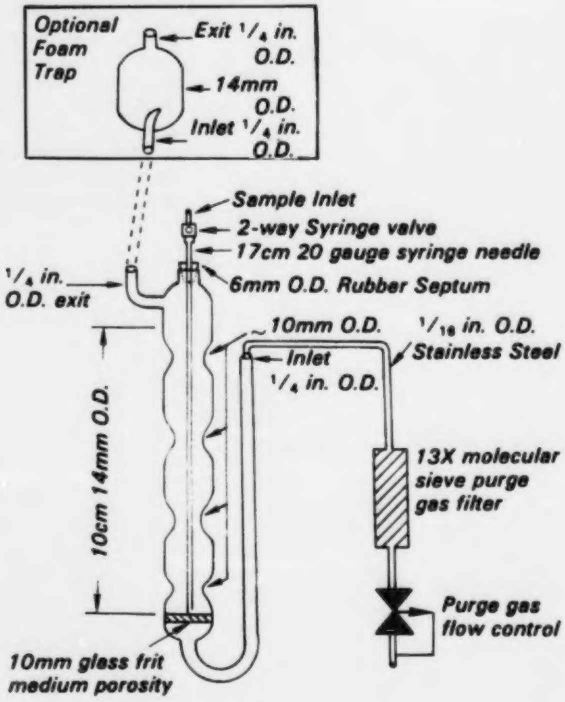

Optional Foam Trap
Exit 1/4 in. O.D.
14mm O.D.
Inlet 1/4 in. O.D.
Sample Inlet
2-way Syringe valve
17cm 20 gauge syringe needle
1/4 in. O.D. exit
6mm O.D. Rubber Septum
10mm O.D.
1/16 in. O.D. Stainless Steel
Inlet 1/4 in. O.D.
10cm 14mm O.D.
13X molecular sieve purge gas filter
Purge gas flow control
10mm glass frit medium porosity

Figure 1. *Purging device*

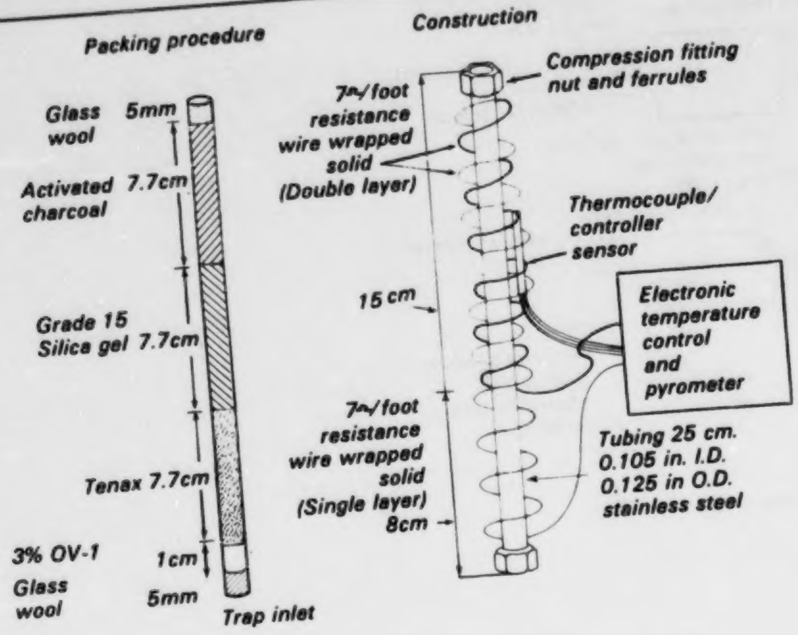

Packing procedure
Construction
Glass wool 5mm
Activated charcoal 7.7cm
Grade 15 Silica gel 7.7cm
Tenax 7.7cm
3% OV-1 1cm
Glass wool 5mm
Trap inlet
7Ω/foot resistance wire wrapped solid (Double layer)
Compression fitting nut and ferrules
Thermocouple/ controller sensor
15 cm
Electronic temperature control and pyrometer
7Ω/foot resistance wire wrapped solid (Single layer)
8cm
Tubing 25 cm. 0.105 in. I.D. 0.125 in O.D. stainless steel

Figure 2. *Trap packings and construction to include desorb capability*

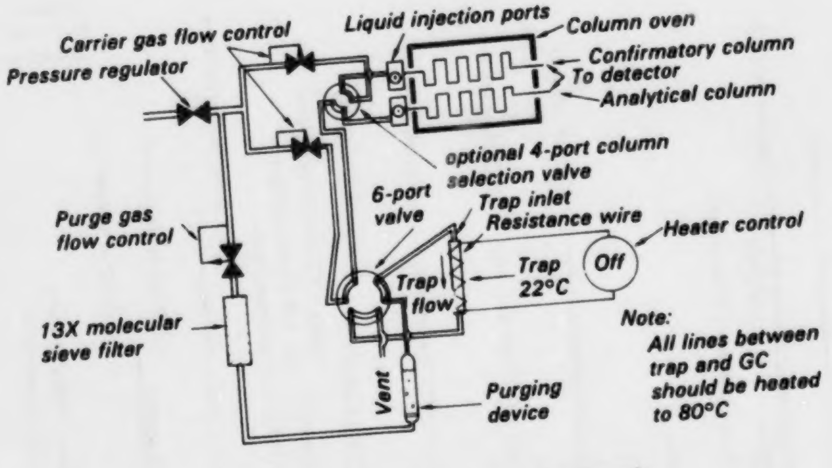

Carrier gas flow control
Liquid injection ports
Column oven
Confirmatory column
To detector
Analytical column
Pressure regulator
optional 4-port column selection valve
6-port valve
Trap inlet
Resistance wire
Heater control
Off
Purge gas flow control
Trap flow
Trap 22°C
13X molecular sieve filter
Vent
Purging device
Note: All lines between trap and GC should be heated to 80°C

Figure 3. *Schematic of purge and trap device — purge mode*

20<

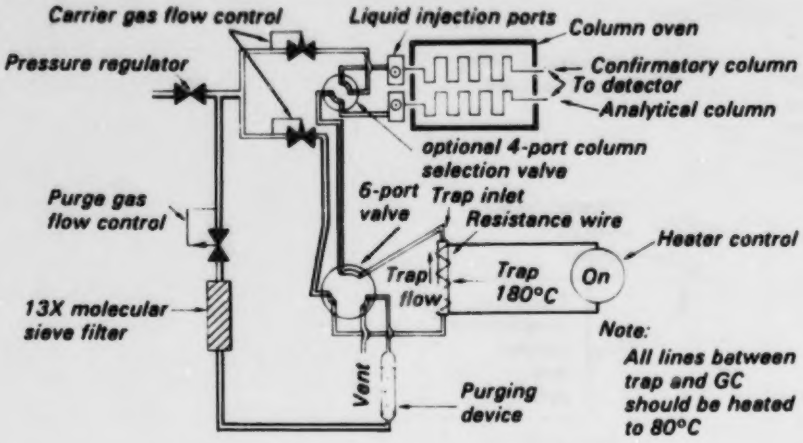
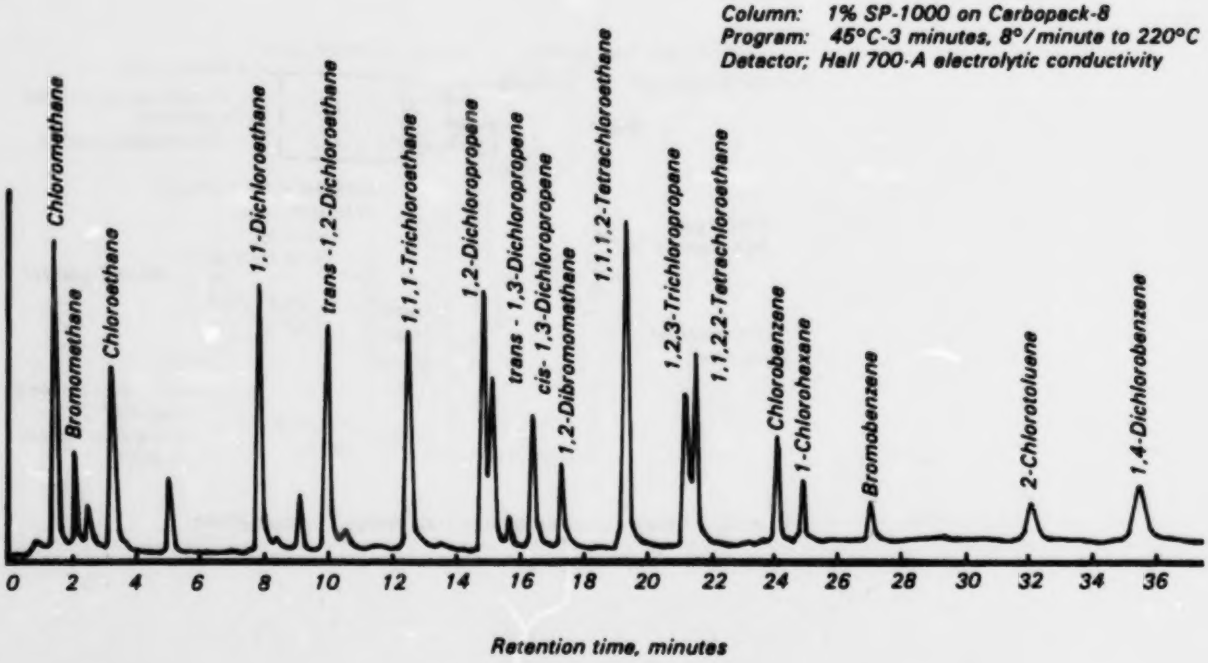

Carrier gas flow control
Liquid injection ports
Column oven
Pressure regulator
Confirmatory column
To detector
Analytical column
optional 4-port column selection valve
6-port valve
Trap inlet
Resistance wire
Purge gas flow control
Heater control
On
Trap flow
Trap 180°C
13X molecular sieve filter
Vent
Purging device
Note:
All lines between trap and GC should be heated to 80°C

Figure 4. *Schematic of purge and trap device — desorb mode*



Column: 1% SP-1000 on Carbopack-B
Program: 45°C-3 minutes, 8°/minute to 220°C
Detector; Hall 700-A electrolytic conductivity

Figure 5. *Gas chromatogram of purgeable halocarbons*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Purgeable Aromatics—Method 602

1. Scope and Application

1.1 This method covers the determination of various purgeable aromatics. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Benzene	34030	71-43-2
Chlorobenzene	34301	108-90-7
1,2-Dichlorobenzene	34536	95-50-1
1,3-Dichlorobenzene	34566	541-73-1
1,4-Dichlorobenzene	34571	106-46-7
Ethylbenzene	34371	100-41-4
Toluene	34010	108-88-3

1.2 This is a purge and trap gas chromatographic method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 624 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above.

1.3 The method detection limit (MDL, defined in Section 12.1(1)) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from these listed depending upon the nature of interferences in the sample matrix.

1.4 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval for alternate test procedures under 40 CFR 136.4 and 136.5

1.5 This method is restricted to use by or under the supervision of analysts experienced in the operation of a purge and trap system and a gas chromatograph and in the interpretation of chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 An inert gas is bubbled through a 5-mL water sample contained in a specially-designed purging chamber at ambient temperature. The aromatics are efficiently transferred from the aqueous phase to the vapor phase. The vapor is swept through a sorbent trap where the aromatics are trapped. After purging is completed, the trap is heated and backflushed with the inert gas to desorb the aromatics onto a gas chromatographic column. The gas chromatograph is temperature programmed to separate the aromatics which are then detected with a photoionization detector(2,3).

2.2 The method provides an optional gas chromatographic column that may be helpful in resolving the compounds of interest from interferences that may occur.

3. Interferences

3.1 Impurities in the purge gas and organic compounds out-gassing from the plumbing ahead of the trap account for the majority of contamination problems. The analytical system must be demonstrated to be free from contamination under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5. The use of non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purging device should be avoided.

3.2 Samples can be contaminated by diffusion of volatile organics through the septum seal into the sample during shipment and storage. A field reagent blank prepared from reagent water and carried through the sampling and handling protocol can serve as a check on such contamination.

3.3 Contamination by carry-over can occur whenever high level and low level samples are sequentially analyzed. To reduce carry-over, the purging device and sample syringe must be rinsed with reagent water between sample analyses. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of reagent water to check for cross contamination. For samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds or high aromatic levels, it may be necessary to wash out the purging device with a detergent solution, rinse it with distilled water, and then dry it in an oven at 105 °C between analyses. The trap and other parts of the system are also subject to contamination; therefore, frequent bakeout and purging of the entire system may be required.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(4-6) for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: benzene and 1,4-dichlorobenzene. Primary standards of these toxic compounds should be prepared in a hood. An NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete sampling.

5.1.1 Vial—25-mL capacity or larger, equipped with a screw cap with hole in center (Pierce #13075 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105 °C before use.

5.1.2 Septum—Teflon-faced silicone (Pierce #12722 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105 °C for one hour before use.

5.2 Purge and trap device—The purge and trap device consists of three separate pieces of equipment: the sample purger, trap, and the desorber. Several complete devices are now commercially available.

5.2.1 The sample purger must be designed to accept 5-mL samples with a water column at least 3 cm deep. The gaseous head space between the water column and the trap must have a total volume of less than 15 mL. The purge gas must pass through the water column as finely divided bubbles with a diameter of less than 3 mm at the origin. The purge gas must be introduced no more than 5 mm from the base of the water column. The sample purger, illustrated in Figure 1, meets these design criteria.

5.2.2 The trap must be at least 25 cm long and have an inside diameter of at least 0.105 inch.

5.2.2.1 The trap is packed with 1 cm of methyl silicone and 23 cm 2,6-diphenylene oxide polymer as shown in Figure 2. This trap was used to develop the method performance statements in Section 12.

5.2.2.2 Alternatively, either of the two traps described in Method 601 may be used, although water vapor will preclude the measurement of low concentrations of benzene.

5.2.3 The desorber must be capable of rapidly heating the trap to 180 °C. The polymer section of the trap should not be heated higher than 180 °C and the remaining sections should not exceed 200 °C. The desorber design, illustrated in Figure 2, meets these criteria.

5.2.4 The purge and trap device may be assembled as a separate unit or be coupled to a gas chromatograph as illustrated in Figures 3, 4, and 5.

5.3 Gas chromatograph—Analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and stripchart recorder. A data system is recommended for measuring peak areas.

5.3.1 Column 1—6 ft long × 0.082 in ID stainless steel or glass, packed with 5% SP-1200 and 1.75% Bentone-34 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements and the MDLs listed in Tables 1 and 2. Guidelines for the use of alternate column packings are provided in Section 10.1.

5.3.2 Column 2—8 ft long × 0.1 in ID stainless steel or glass, packed with 5% 1,2,3-Tris(2-cyanoethoxy)propane on Chromosorb W-AW (60/80 mesh) or equivalent.

5.3.3 Detector—Photoionization detector (h-nu Systems, Inc. Model PI-51-02 or equivalent). This type of detector has been proven effective in the analysis of wastewaters for the parameters listed in the scope, and was used to develop the performance statements in Section 12. Guidelines for the use of alternate detectors are provided in Section 10.1.

5.4 Syringes—5-mL glass hypodermic with Luerlok tip (two each), if applicable to the purge device.

5.5 Micro syringes—25 µL, 0.006 in ID needle.

5.6 Syringe valve—2-way, with Luer ends (three each).

5.7 Bottle—15-mL screw-cap with Teflon cap liner.

5.8 Balance—Analytical, capable of accurately weighing 0.0001 g.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of the parameters of interest.

6.1.1 Reagent water can be generated by passing tap water through a carbon filter bed containing about 1 lb. of activated carbon. (Filtrasorb-300 or equivalent (Calgon Corp.)).

6.1.2 A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

6.1.3 Reagent water may also be prepared by boiling water for 15 minutes. Subsequently, while maintaining the temperature at 90 °C, bubble a contaminant-free inert gas through the water for one hour. While still hot, transfer the water to a narrow mouth screw-cap bottle and seal with a Teflon-lined septum and cap.

6.2 Sodium thiosulfate—(ACS) Granular.

6.3 Hydrochloric acid (1 + 1)—Add 50 mL of concentrated HCl to 50 mL of reagent water.

6.4 Trap Materials

6.4.1 2,6-Diphenylene oxide polymer-Tenax, (60/80 mesh) chromatographic grade or equivalent.

6.4.2 Methyl silicone—3% OV-1 on Chromosorb-W (60/80 mesh) or equivalent.

6.5 Methyl alcohol—Pesticide quality or equivalent.

6.6 Stock standard solutions—Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions. Prepare stock standard solutions in methyl alcohol using assayed liquids. Because benzene and 1,4-dichlorobenzene are suspected carcinogens, primary dilutions of these materials should be prepared in a hood.

6.6.1 Place about 9.8 mL of methyl alcohol into a 10-mL ground glass stoppered volumetric flask. Allow the flask to stand, unstoppered, for about 10 minutes or until all alcohol wetted surfaces have dried. Weigh the flask to the nearest 0.1 mg.

6.6.2 Using a 100-µL syringe, immediately add two or more drops of assayed reference material to the flask, then reweigh. Be sure that the drops fall directly into the alcohol without contacting the neck of the flask.

6.6.3 Reweigh, dilute to volume, stopper, then mix by inverting the flask several times. Calculate the concentration in micrograms per microliter from the net gain in weight. When compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used, at any concentration, if they are certified by the manufacturer or by an independent source.

6.6.4 Transfer the stock standard solution into a Teflon-sealed screw-cap bottle. Store at 4 °C and protect from light.

6.6.5 All standards must be replaced after one month, or sooner if comparison with check standards indicate a problem.

6.7 Secondary dilution standards—Using stock standard solutions, prepare secondary dilution standards in methyl alcohol that contain the compounds of interest, either singly or mixed together. The secondary dilution standards should be prepared at concentrations such that the aqueous calibration standards prepared in Sections 7.3.1 or 7.4.1 will bracket the working range of the analytical system. Secondary solution standards must be stored with zero headspace and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, in Cincinnati, Ohio.

7. Calibration

7.1 Assemble a purge and trap device that meets the specifications in Section 5.2. Condition the trap overnight at 180 °C by backflushing with an inert gas flow of at least 20 mL/min. Prior to use, daily condition traps 10 minutes while backflushing at 180 °C.

7.2 Connect the purge and trap device to a gas chromatograph. The gas chromatograph must be operated using temperature and flow rate parameters equivalent to those in Table 1. Calibrate the purge and trap-gas chromatographic system using either the external standard technique (Section 7.3) or the internal standard technique (Section 7.4.).

7.3 External standard calibration procedure:

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter by carefully adding 20.0 µL of one or more secondary dilution standards to 100, 500, or 1000 mL of reagent water. A 25-µL syringe with a 0.006 inch ID needle should be used for this operation. One of the external standards should be at a concentration near, but above, the MDL (see Table 1) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector. These aqueous standards must be prepared fresh daily.

7.3.2 Analyze each calibration standard according to Section 10, and tabulate peak height or area responses versus the concentration in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range ($<10\%$ relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.3.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.4 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples. The compound, α,α,α-trifluorotoluene, recommended as a surrogate spiking compound in Section 8.7 has been used successfully as an internal standard.

7.4.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest as described in Section 7.3.1.

7.4.2 Prepare a spiking solution containing each of the internal standards using the procedures described in Sections 6.6 and 6.7. It is recommended that the secondary dilution standard be prepared at a concentration of 15 μg/mL of each internal standard compound. The addition of 10 μL of this standard to 5.0 mL of sample or calibration standard would be equivalent to 30 μg/L.

7.4.3 Analyze each calibration standard, according to Section 10, adding 10 μL of internal standard spiking solution directly to the syringe as indicated in Section 10.4. Tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

Eq. 1 $RF = (A_sC_{is})/(A_{is}C_s)$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard.

C_s = Concentration of the parameter to be measured.

If the RF value over the working range is a constant ($<$10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.4.4 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methyl alcohol 500 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a syringe, add 10 μL of the check sample concentrate to each of a minimum of four 5-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X - R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (LCL) = R − 3s

where R and s are calculated as in Section 8.2.3

The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly[7].

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 11.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Each day, the analyst must demonstrate through the analysis of reagent water, that interferences from the analytical system are under control.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

8.7 The analyst should maintain constant surveillance of both the performance of the analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and blank with surrogate compounds (e.g. *a,a,a,*-trifluorotoluene). From stock standard solutions prepared as above, add a volume to give 7500 µg of each surrogate to 45 mL of organic-free water contained in a 50-mL volumetric flask, mix and dilute to volume (15 ng/µL). If the internal standard calibration procedure is being used, the surrogate compounds may be added directly to the internal standard spiking solution (Section 7.4.2). Dose 10 µL of this surrogate spiking solution directly into the 5-mL syringe with every sample and reference standard analyzed. Prepare a fresh surrogate spiking solution on a weekly basis.

9. Sample Collection, Preservation, and Handling

9.1 The samples must be iced or refrigerated from the time of collection until extraction. If the sample contains free or combined chlorine, add sodium thiosulfate preservative (10 mg/40 mL is sufficient for up to 5 ppm Cl_2) to the empty sample bottles just prior to shipping to the sampling site. USEPA Methods 330.4 or 330.5 may be used to measure residual chlorine(8). Field Test Kits are available for this purpose.

9.2 Collect about 500 mL sample in a clean container. Adjust the pH of the sample to about 2 by adding 1 + 1 HCl while stirring gently. Fill the sample bottle in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottle so that no air bubbles are entrapped in it. Maintain the hermetic seal on the sample bottle until time of analysis.

9.3 All samples must be analyzed within 14 days of collection.(3)

10. Sample Extraction and Gas Chromatography

10.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and method detection limits that can be achieved by this method. An example of the separations achieved by Column 1 is shown in Figure 6. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

10.2 Calibrate the system daily as described in Section 7.

10.3 Adjust the purge gas (nitrogen or helium) flow rate to 40 mL/min. Attach the trap inlet to the purging device, and set the device to purge. Open the syringe valve located on the purging device sample introduction needle.

10.4 Allow sample to come to ambient temperature prior to introducing it into the syringe. Remove the plunger from a 5-mL syringe and attach a closed syringe valve. Open the sample bottle (or standard) and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. Since this process of taking an aliquot destroys the validity of the sample for future analysis, the analyst should fill a second syringe at this time to protect against possible loss of data. Add 10.0 µL of the surrogate spiking solution (Section 8.7) and 10.0 µL of the internal standard spiking solution (Section 7.4.2), if applicable, through the valve bore, then close the valve.

10.5 Attach the syringe-syringe valve assembly to the syringe valve on the purging device. Open the syringe valves and inject the sample into the purging chamber.

10.6 Close both valves and purge the sample for 12.0 ± 0.1 minutes at ambient temperature.

10.7 After the 12-minute purge time, disconnect the purge chamber from the trap. Dry the trap by maintaining a flow of 40 mL/min of dry purge gas through it for six minutes. See Figure 4. A dry purger should be inserted into the device to minimize moisture in the gas. Attach the trap to the chromatograph, adjust the device to the desorb mode, and begin to temperature program the gas chromatograph. Introduce the trapped materials to the GC column by rapidly heating the trap to 180 °C while backflushing the trap with an inert gas between 20 and 60 mL/min for four minutes. If rapid heating cannot be achieved, the gas chromatographic column must be used as a secondary trap by cooling it to 30 °C (subambient temperature, if poor peak geometry and random retention time problems persist) instead of the initial program temperature of 50 °C.

10.8 While the trap is being desorbed onto the GC column, empty the purging chamber using the sample introduction syringe. Wash the chamber with two 5-mL flushes of reagent water.

10.9 After desorbing the sample for four minutes, recondition the trap by returning the purge and trap device to the purge mode. Wait 15 seconds then close the syringe valve on the purging device to begin gas flow through the trap. The trap temperature should be maintained at 180 °C. After approximately seven minutes, turn off the trap heater and open the syringe valve to stop the gas flow through the trap. When cool, the trap is ready for the next sample.

10.10 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

10.11 If the response for the peak exceeds the working range of the system, prepare a dilution of the sample with reagent water from the aliquot in the second syringe and reanalyze.

11. Calculations

11.1 Determine the concentration of individual compounds in the sample.

11.1.1 If the external standard calibration procedure is used, calculate the concentration of material from the peak response using the calibration curve or calibration factor determined in Section 7.3.2.

11.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.4.3 and equation 2.

Eq. 2.
Concentration $\mu g/L = (A_sC_{is})/(A_{is})(RF)$
where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard.

11.2 Report results in micrograms per liter. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

11.3 For samples processed as part of a set where the spiked sample recovery falls outside of the control limits which were described in Section 8.3, data for the affected parameters must be labeled as suspect.

12. Method Performance

12.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(9). Similar results were achieved using representative wastewaters.

12.2 This method has been demonstrated to be applicable for the concentration range from the MDL up to 1000 × MDL(9). Direct aqueous injection techniques should be used to measure concentration levels above 1000 × MDL.

12.3 In a single laboratory (Monsanto Research), using reagent water and wastewaters spiked at or near background levels, the average recoveries presented in Table 2 were obtained(9). The standard deviation of the measurement in percent recovery is also included in Table 2.

12.4 The Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. Bellar, T.A., and Lichtenberg, J.J. *Journal American Water Works Association, 66,* 739, (1974).
3. Bellar, T.A., and Lichtenberg, J.J. "Semi-Automated Headspace Analysis of Drinking Waters and Industrial Waters for Purgeable Volatile Organic Compunds," Proceedings of Symposium on Measurement of Organic Pollutants in Water and Wastewater. American Society for Testing and Materials, STP 686, C.E. Van Hall, editor, 1978.
4. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health. Publication No. 77-206, August 1977.
5. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Safety, 3rd Edition, 1979.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268. March 1979.
8. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268. March 1979.
9. "EPA Method Validation Study 24, Method 602 (Purgeable Aromatics)," Report for EPA Contract 68-03-2856 (In preparation).

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	Retention Time (min.) Column 1	Retention Time (min.) Column 2	Method Detection Limit µg/L
Benzene	3.33	2.75	0.2
Toluene	5.75	4.25	0.2
Ethylbenzene	8.25	6.25	0.2
Chlorobenzene	9.17	8.02	0.2
1,4-Dichlorobenzene	16.8	16.2	0.3
1,3-Dichlorobenzene	18.2	15.0	0.4
1,2-Dichlorobenzene	25.9	19.4	0.4

Column 1 conditions: Supelcoport 100/120 mesh coated with 5% SP-1200 and 1.75% Bentone-34 packed in a 6 ft. × 0.085 in ID stainless steel column with helium carrier gas at 36 cc/min flow rate. Column temperature held at 50°C for 2 min. then programmed at 6°C/min to 90°C for a final hold.

Column 2 conditions: Chromosorb W-AW 60/80 mesh coated with 5% 1,2,3-Tris(2-cyanoethyoxy)propane packed in a 6 ft. × 0.085 in ID stainless steel column with helium carrier gas at 30 cc/min flow rate. Column temperature held at 40°C for 2 min then programmed at 2°C/min to 100°C for a final hold.

Table 2. *Single Operator Accuracy and Precision*

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
Benzene	91	10.0	0.5-9.7	21	3
Chlorobenzene	97	9.4	0.5-100	21	3
1,2-Dichlorobenzene	104	27.7	0.5-10.0	21	3
1,3-Dichlorobenzene	97	20.0	0.5-4.8	21	3
1,4-Dichlorobenzene	120	20.4	0.5-10.0	21	3
Ethylbenzene	98	12.4	0.5-9.9	21	3
Toluene	77	12.1	0.5-100	21	3

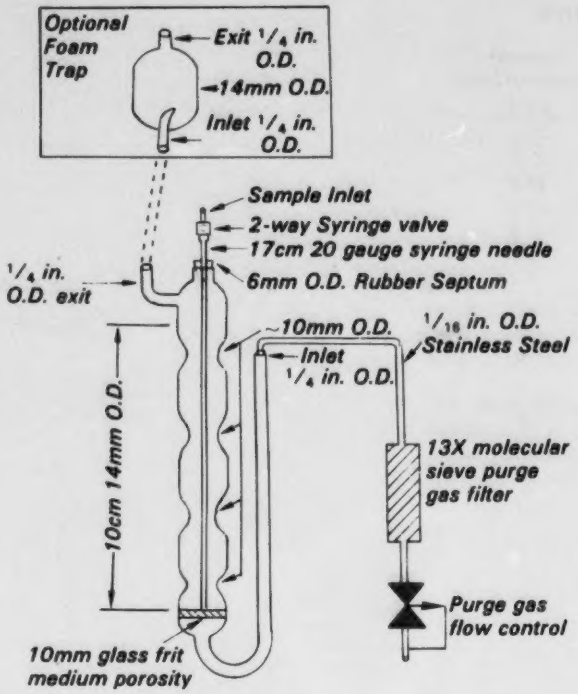

Optional
Foam
Trap
Exit 1/4 in. O.D.
14mm O.D.
Inlet 1/4 in. O.D.
Sample Inlet
2-way Syringe valve
17cm 20 gauge syringe needle
1/4 in. O.D. exit
6mm O.D. Rubber Septum
~10mm O.D.
1/16 in. O.D. Stainless Steel
Inlet 1/4 in. O.D.
10cm 14mm O.D.
13X molecular sieve purge gas filter
Purge gas flow control
10mm glass frit medium porosity

Figure 1. *Purging device*

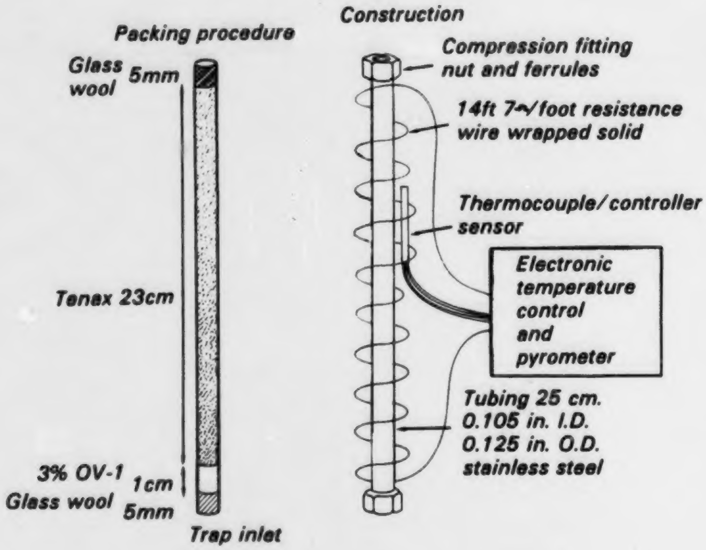

Packing procedure
Construction
Glass wool 5mm
Tenax 23cm
3% OV-1 1cm
Glass wool 5mm
Trap inlet
Compression fitting nut and ferrules
14ft 7Ω/foot resistance wire wrapped solid
Thermocouple/controller sensor
Electronic temperature control and pyrometer
Tubing 25 cm. 0.105 in. I.D. 0.125 in. O.D. stainless steel

Figure 2. *Trap packings and construction to include desorb capability*

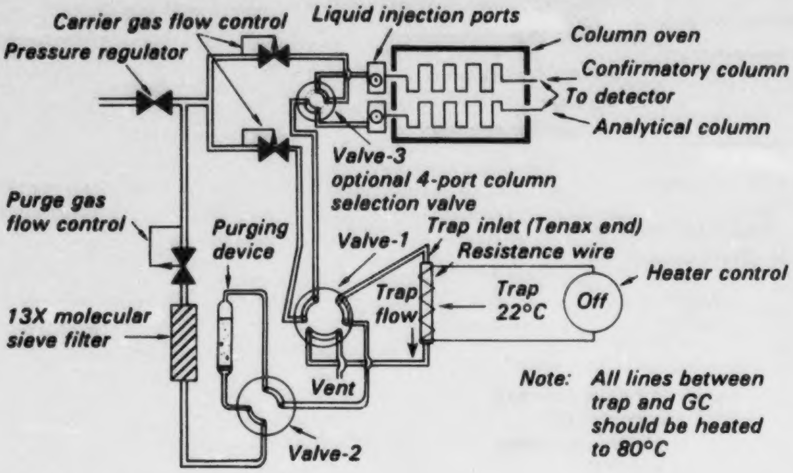

Carrier gas flow control
Liquid injection ports
Column oven
Pressure regulator
Confirmatory column
To detector
Analytical column
Valve-3
optional 4-port column selection valve
Purge gas flow control
Purging device
Valve-1
Trap inlet (Tenax end)
Resistance wire
Heater control
Trap flow
Trap 22°C
Off
13X molecular sieve filter
Vent
Valve-2
Note: All lines between trap and GC should be heated to 80°C

Figure 3. *Purge-trap system (Purge-sorb Mode)*

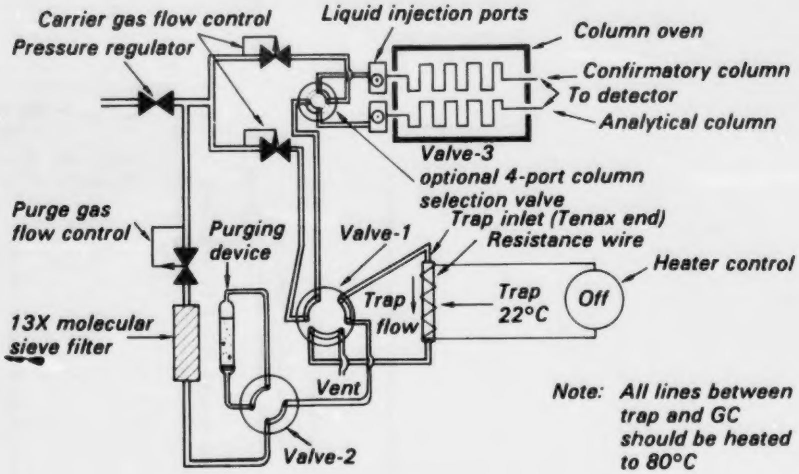

Carrier gas flow control
Liquid injection ports
Column oven
Pressure regulator
Confirmatory column
To detector
Analytical column
Valve-3
optional 4-port column selection valve
Purge gas flow control
Purging device
Valve-1
Trap inlet (Tenax end)
Resistance wire
Heater control
Trap flow
Trap 22°C
Off
13X molecular sieve filter
Vent
Valve-2
Note: All lines between trap and GC should be heated to 80°C

Figure 4. *Purge-trap system (Trap-dry Mode).*

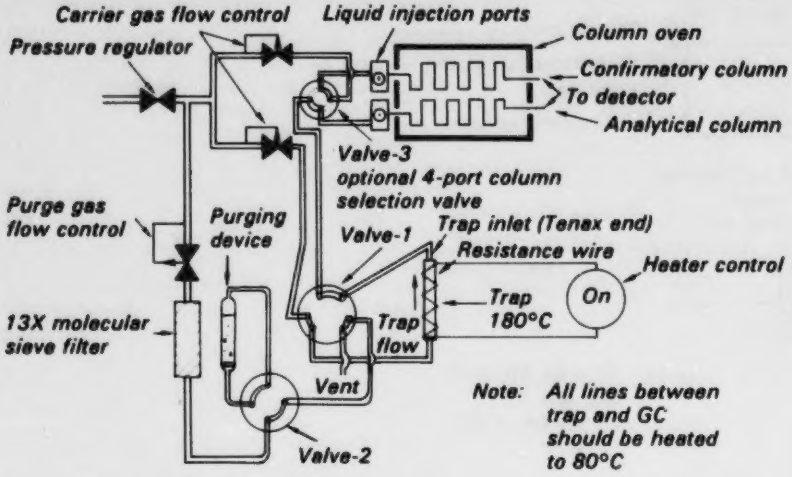

Carrier gas flow control
Liquid injection ports
Column oven
Pressure regulator
Confirmatory column
To detector
Analytical column
Valve-3
optional 4-port column
selection valve
Purge gas
flow control
Purging
device
Valve-1
Trap inlet (Tenax end)
Resistance wire
Heater control
On
Trap
180°C
Trap
flow
13X molecular
sieve filter
Vent
Valve-2
Note: All lines between
trap and GC
should be heated
to 80°C

Figure 5. *Purge-trap system (Desorb Mode).*

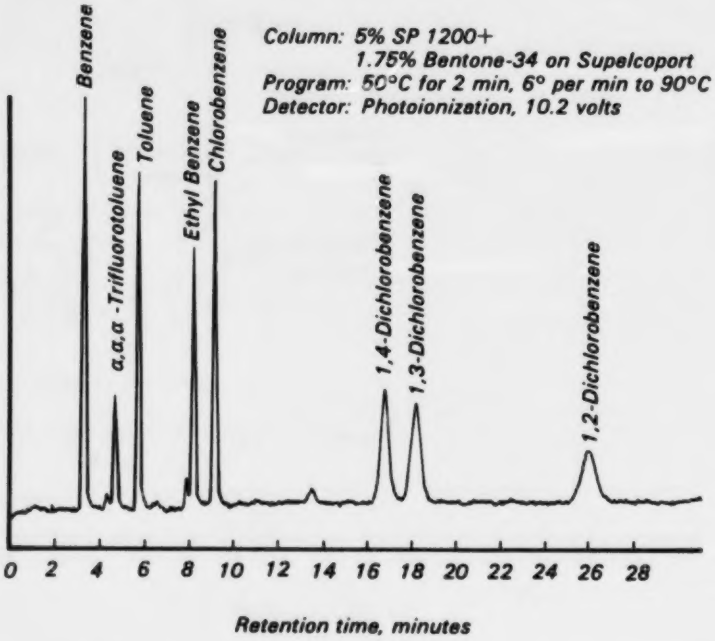


Figure 6. *Gas chromatogram of purgeable aromatics.*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Acrolein and Acrylonitrile—Method 603

1. Scope and Application

1.1 This method covers the determination of acrolein and acrylonitrile. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Acrolein	34210	107-02-8
Acrylonitrile	34215	107-13-1

1.2 This is a purge and trap gas chromatographic method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for either or both of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 624 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for the parameters listed above, if used with the purge and trap conditions described in this method.

1.3 The method detection limit (MDL, defined in Section 12.1)[1] for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5

1.5 This method is restricted to use by or under the supervision of analysts experienced in the operation of a purge and trap system and a gas chromatograph (GC) and in the interpretation of GC chromatograms. Each analyst must demonstrate the abilitv to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 An inert gas is bubbled through a 5-mL water sample contained in a heated purging chamber. Acrolein and acrylonitrile are transferred from the aqueous phase to the vapor phase. The vapor is swept through a sorbent trap where the analytes are trapped. After the purge is completed, the trap is heated and backflushed with the inert gas to desorb the compounds onto a gas chromatographic column. The gas chromatograph is temperature programmed to separate the analytes which are then detected with a flame ionization detector (2,3).

2.2 The method provides an optional gas chromatographic column that may be helpful in resolving the compounds of interest from the interferences that may occur.

3. Interferences

3.1 Impurities in the purge gas and organic compounds out-gassing from the plumbing ahead of the trap account for the majority of contamination problems. The analytical system must be demonstrated to be free from contamination under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5. The use of non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purging device should be avoided.

3.2 Samples can be contaminated by diffusion of volatile organics through the septum seal into the sample during shipment and storage. A field reagent blank prepared from reagent water and carried through the sampling and handling protocol can serve as a check on such contamination.

3.3 Contamination by carry-over can occur whenever high level and low level samples are sequentially analyzed. To reduce carry-over, the purging device and sample syringe must be rinsed out between samples with reagent water. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of reagent water to check for cross contamination. For samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds or high analyte levels, it may be necessary to wash out the purging device with a detergent solution, rinse it with distilled water, and then dry it in a 105 °C oven between analyses. The trap and other parts of the system are also subject to contamination, therefore, frequent bakeout and purging of the entire system may be required.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(4-6) for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete sampling.

5.1.1 Vial—25-mL capacity or larger, equipped with a screw cap with a hole in the center (Pierce #13075 or equivalent). Detergent wash, rinse with tap and distilled water and dry at 105 °C before use.

5.1.2 Septum—Teflon-faced silicone (Pierce #12722 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105 °C for one hour before use.

5.2 Purge and trap device—The purge and trap device consists of three separate pieces of equipment: the sample purger, the trap, and the desorber. Several complete devices are now commercially available.

5.2.1 The sample purger must be designed to accept 5-mL samples with a water column at least 3 cm deep. The gaseous head space between the water column and the trap must have a total volume of less than 15 mL. The purge gas must pass through the water column as finely divided bubbles with a diameter of less than 3 mm at the origin. The purge gas must be introduced no more than 5 mm from the base of the water column. The purge device must be capable of being heated to 85 °C within 3.0 minutes after transfer of the sample to the purge device and being held at 85 ± 2 °C during purge cycle. The entire water column in the purge device must be heated. Design of this modification to the standard purge device is optional, however, use of a water bath is suggested.

5.2.1.1 Heating mantle—To be used to heat water bath.

5.2.1.2 Temperature controller—equipped with thermocouple/sensor to accurately control water bath temperature to ± 2 °C. The sample purger illustrated in Figure 1 meets these design criteria.

5.2.2 The trap must be at least 25 cm long and have an inside diameter of at least 0.105 inch. The trap must be packed to contain 1.0 cm of methyl silicone coated packing (Section 6.5.2) and 23 cm of 2,6-diphenylene oxide polymer (Section 6.5.1). The minimum specifications for the trap are illustrated in Figure 2.

5.2.3 The desorber must be capable of rapidly heating the trap to 100 °C. The polymer section of the trap should not be heated higher than 180 °C. The desorber, illustrated in Figure 2, meets these design criteria.

5.2.4 The purge and trap device may be assembled as a separate unit as illustrated in Figure 3 or be coupled to a gas chromatograph.

5.3 pH paper—Narrow pH range, about 3.5 to 5.5 (Fisher Scientific Short Range Alkacid No. 2, #14-837-2 or equivalent).

5.4 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.4.1 Column 1—6 ft long × 0.1 in ID stainless steel or glass, packed with Durapak-Carbowax 400/Porasil-C (100/120 mesh) or equivalent. This column was used to develop the method performance statements given in Section 12. Guidelines for the use of alternate column packings are provided in Section 10.1.

5.4.2 Column 2—6 ft long × 0.1 in ID stainless steel or glass, packed with Chromosorb 101 (60/80 mesh) or equivalent.

5.4.3 Detector—Flame ionization. This type of detector has proven effective in the analysis of wastewaters for the parameters listed in the scope, and was used to develop the method performance statements in Section 12. Guidelines for the use of alternate detectors are provided in Section 10.1.

5.5 Syringes—5-mL, glass hypodermic with Luerlok tip (two each).

5.6 Micro syringes—25 µL.

5.7 Syringe valve, 2-way with Luer ends (three each).

5.8 Bottle—15-mL screw-cap with Teflon cap liner.

5.9 Balance—Analytical, capable of accurately weighing 0.0001 g.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of the parameters of interest.

6.1.1 Reagent water can be generated by passing tap water through a carbon filter bed containing about one pound of activated carbon (Filtrasorb-300 or equivalent, Calgon Corp).

6.1.2 A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

6.1.3 Reagent water may also be prepared by boiling water for 15 minutes. Subsequently, while maintaining the temperature at 90 °C, bubble a contaminant-free inert gas through the water for one hour. While still hot, transfer the water to a narrow mouth screw cap bottle and seal with a Teflon lined septum and cap.

6.2 Sodium thiosulfate—(ACS) Granular.

6.3 Sodium hydroxide solution (10 N)—Dissolve 40 g NaOH in reagent water and dilute to 100 mL.

6.4 Hydrochloric acid solution (1 + 1)—Slowly add 50 mL concentrated HCl to 50 mL reagent water.

6.5 Trap Materials

6.5.1 2,6-Diphenylene oxide polymer, Tenax (60/80 mesh) chromatographic grade.

6.5.2 Methyl Silicone packing—3% OV-1 on Chromosorb-W (60/80 mesh) or equivalent.

6.6 Stock standard solutions—Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions. Prepare stock standard solutions in reagent water using assayed liquids. Since acrolein and acrylonitrile are lachrymators, primary dilutions of these compounds should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be used when the analyst handles high concentrations of such materials.

6.6.1 Place about 9.8 mL of reagent water into a 10-mL ground glass stoppered volumetric flask. For acrolein standards the reagent water must be adjusted to pH 4 to 5. Weigh the flask to the nearest 0.1 mg.

6.6.2 Using a 100-μL syringe, immediately add two or more drops of assayed reference material to the flask, then reweigh. Be sure that the drops fall directly into the water without contacting the neck of the flask.

6.6.3 Reweigh, dilute to volume, stopper, then mix by inverting the flask several times. Calculate the concentration in micrograms per microliter from the net gain in weight. When compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.6.4 Transfer the stock standard solution into a Teflon-sealed screw-cap bottle. Store at 4 °C and protect from light.

6.6.5 Prepare fresh standards weekly.

6.7 Secondary dilution standards—Using stock standard solutions, prepare secondary dilution standards in reagent water that contain the compounds of interest, either singly or mixed together. The secondary dilution standards should be prepared at concentrations such that the aqueous calibration standards prepared in Sections 7.3.1 or 7.4.1 will bracket the working range of the analytical system. Secondary dilution standards should be prepared weekly and stored at 4 °C. They should be checked frequently for signs of degradation or evaporation. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, in Cincinnati, Ohio.

7. Calibration

7.1 Assemble a purge and trap device that meets the specifications in Section 5.2. Condition the trap overnight at 180 °C by backflushing with an inert gas flow of at least 20 mL/min. Prior to use, daily condition traps 10 minutes while backflushing at 180 °C.

7.2 Connect the purge and trap device to a gas chromatograph. The gas chromatograph must be operated using temperature and flow rate parameters equivalent to those in Table 1. Calibrate the purge and trap-gas chromatographic system using either the external standard technique (Section 7.3) or the internal standard technique (Section 7.4).

7.3 External standard calibration procedure:

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter by carefully adding 20.0 μL of one or more secondary dilution standards to 100, 500, or 1000 mL of reagent water. A 25-μL syringe should be used for this operation. One of the external standards should be at a concentration near, but above, the method detection limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector. These standards must be prepared fresh daily.

7.3.2 Analyze each calibration standard according to Section 10, and tabulate peak height or area responses versus the concentration of the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.3.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.4 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.4.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest as described in Section 7.3.1.

7.4.2 Prepare a spiking solution containing each of the internal standards using the procedures described in Sections 6.6 and 6.7. It is recommended that the secondary dilution standard be prepared at a concentration of 15 μg/mL of each internal standard compound. The addition of 10 μL of this standard to 5.0 mL of sample or calibration standard would be equivalent to 30 μg/L.

7.4.3 Analyze each calibration standard according to Section 10 adding 10 L of internal standard spiking solution directly to the syringe (Section 10.4). Tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

Eq. 1 RF = $(A_sC_{is})/(A_{is}C_s)$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard.

C_s = Concentration of the parameter to be measured.

If the RF value over the working range is a constant ($<$10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.4.4 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in reagent water 500 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a syringe, add 10 μL of the check sample concentrate to each of a minimum of four 5-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X - R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (LCL) = R − 3s

where R and s are calculated as in Sections 8.2.3.

The UCL and LCL can be used to construct control charts(7) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(7).

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 11.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Each day, the analyst must demonstrate through the analysis of reagent water, that interferences from the analytical system are under control.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 The samples must be iced or refrigerated from the time of collection until extraction. If the sample contains free or combined chlorine, add sodium thiosulfate preservative (10 mg/40 mL is sufficient for up to 5 ppm Cl_2) to the empty sample bottles just prior to shipping to the sampling site. EPA methods 330.4 and 330.5 may be used for measurement of chlorine residual(8). Field test kits are available for this purpose.

9.2 If acrolein is to be analyzed, collect about 500 mL sample in a clean glass container. Adjust the pH of the sample to 4 to 5 using acid or base, measuring with narrow range pH paper. Samples for acrolein analysis receiving no pH adjustment must be analyze within three days of sampling.

9.3. Fill a sample bottle just to overflowing in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottle so that no air bubbles are entrapped in it. If preservative has been added, shake vigorously for one minute. Maintain the hermetic seal on the sample bottle until time of analysis.

9.4 All samples must be analyzed within 14 days of collection(3).

10. Sample Extraction and Gas Chromatography

10.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this Table are estimated retention times and MDL that can be achieved by this method. An example of the separations achieved by Column 1 is shown in Figure 4. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met.

10.2 Calibrate the system daily as described in Section 7.

10.3 Adjust the purge gas (nitrogen or helium) flow rate to 20 mL/min. Attach the trap inlet to the purging device, and set the device to purge. Open the syringe valve located on the purging device sample introduction needle.

10.4 Remove the plunger from a 5-mL syringe and attach a closed syringe valve. Open the sample bottle (or standard) and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. Since this process of taking an aliquot destroys the validity of the sample for future analysis, the analyst should fill a second syringe at this time to protect against possible loss of data. Add 10.0 uL of the internal standard spiking solution (Section 7.4.2), if applicable, through the valve bore then close the valve.

10.5 Attach the syringe-syringe valve assembly to the syringe valve on the purging device. Open the syringe valves and inject the sample into the purging chamber.

10.6 Close both valves and purge the sample for 15.0 ± 0.1 minutes while heating at 85 ± 2 °C.

10.7 After the 15-minute purge time, attach the trap to the chromatograph, and adjust the device to the desorb mode. Begin the temperature program for the gas chromatograph. Introduce the trapped materials to the GC column by rapidly heating the trap to 100 ± 10 °C while backflushing the trap with an inert gas between 20 and 60 mL/min for two minutes.

10.8 While the trap is being desorbed into the gas chromatograph, empty the purging chamber using the sample introduction syringe. Wash the chamber with two 5-mL flushes of reagent water.

10.9 After desorbing the sample for 2.0 minutes recondition the trap by returning the purge and trap device to the purge mode. Wait 15 seconds then close the syringe valve on the purging device to begin gas flow through the trap. The trap temperature should be maintained at 100 °C. After approximately seven minutes turn off the trap heater and open the syringe valve to stop the gas flow through the trap. When cool the trap is ready for the next sample.

10.10 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11. Calculations

11.1 Determine the concentration of individual compounds in the sample.

11.1.1 If the external sample calibration procedure is used, calculate the concentration of material from the peak response using the calibration curve or calibration factor determined in Section 7.3.2.

11.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.4.2 and the equation 2:

Eq. 2

Concentration $\mu g/L = (A_s C_{is})/(A_{is})(RF)$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard.

11.2 Report results in micrograms per liter. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

11.3 For samples processed as part of a set where the spiked sample recovery falls outside of the control limits in 8.3, data for the affected parameters must be labeled as suspect.

12. Method Performance

12.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(9). Similar results were achieved using representative wastewaters.

12.2 This method is recommended for the concentration range from the MDL up to 1000 × MDL. Direct aqueous injection techniques should be used to measure concentration levels above 1000 × MDL.

12.3 In a single laboratory (EMSL-CI), using spiked wastewater, the average recoveries presented in Table 2 were obtained(3). Seven replicate spiked samples were analyzed for each parameter. The relative standard deviation of the measurement is also included in Table 2.

12.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. Bellar, T.A., and Lichtenberg, J.J. *Journal American Water Works Association, 66,* 739, (1974).
3. Kerns, E.H., et al, "Determination of Acrolein and Acrylonitrile in Water by Heated Purge and Trap Technique," 1980, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.
4. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health. Publication No. 77-206, Aug. 1977.
5. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory—Cincinnati, Ohio 45268, March 1979.
8. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory—Cincinnati, Ohio 45268. March 1979.
9. "EPA Method Validation Study 25, Method 603 (Acrolein and Acrylonitrile)," Report for EPA Contract 68-03-2856 (In preparation).

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	*Retention Time (min.)* *Column 1*	*Column 2*	*Method Detection Limit μg/L*
Acrolein	*9.2*	*8.2*	*0.6*
Acrylonitrile	*13.5*	*9.8*	*0.5*

Method detection limit based upon recovery of 5.0 μg/L dose into tap water.

Column 1 conditions: Durapak Carbowax 400/Porasil C, (100/120 mesh) packed in a 6 ft × 0.1 in. ID stainless steel or glass column with helium carrier gas at 30 mL/min flow rate. Column temperature held at 45°C for 2 min, then programmed at 8°C/min to 85°C and held for 12 min. The column temperature should then be raised to 120°C for 7 minutes to bake out water. Failure to dry the column may lead to irreproducable retention times(9).

Column 2 conditions: Chromosorb 101, (60/80 mesh) packed in a 6 ft x 0.1 in ID stainless steel, glass column with helium carrier gas at 40 mL/min flow rate. Column temperature held at 80°C for 4 min. then programmed at 15°C/min to 120°C and held 12 min.

Table 2. *Single Operator Accuracy and Precision*

Parameter	*Average Percent Recovery*	*Standard Deviation %*	*Spike Range (μg/L)*	*Number of Analyses*	*Matrix Types*
Acrolein	*96*	*11.6*	*20*	*7*	*1*
Acrylonitrile	*107*	*5.6*	*20*	*7*	*1*

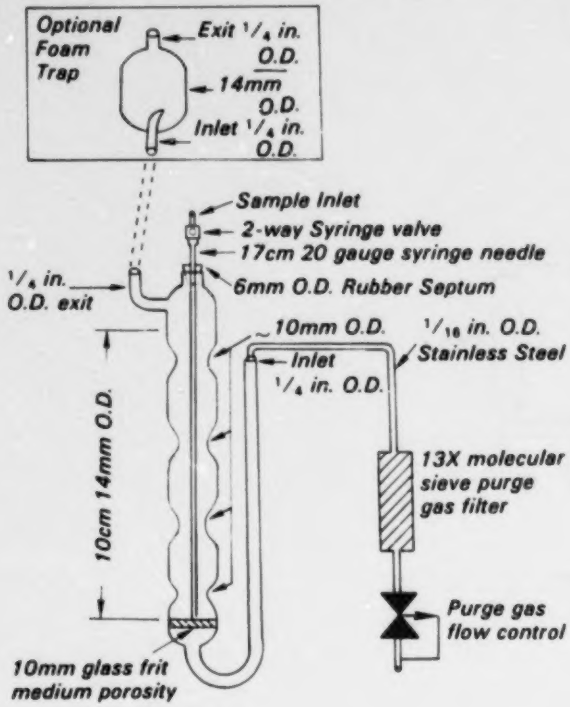

Optional Foam Trap
Exit 1/4 in. O.D.
14mm O.D.
Inlet 1/4 in. O.D.
Sample Inlet
2-way Syringe valve
17cm 20 gauge syringe needle
1/4 in. O.D. exit
6mm O.D. Rubber Septum
10mm O.D.
1/16 in. O.D. Stainless Steel
Inlet 1/4 in. O.D.
10cm 14mm O.D.
13X molecular sieve purge gas filter
Purge gas flow control
10mm glass frit medium porosity

Figure 1. *Purging device*

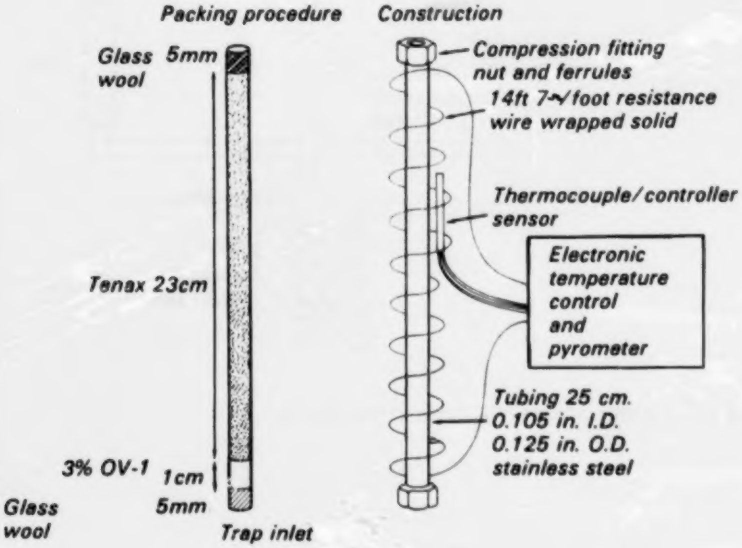

Packing procedure
Construction
Glass wool 5mm
Tenax 23cm
3% OV-1 1cm
Glass wool 5mm
Trap inlet
Compression fitting nut and ferrules
14ft 7Ω/foot resistance wire wrapped solid
Thermocouple/controller sensor
Electronic temperature control and pyrometer
Tubing 25 cm. 0.105 in. I.D. 0.125 in. O.D. stainless steel

Figure 2. *Trap packings and construction to include desorb capability*

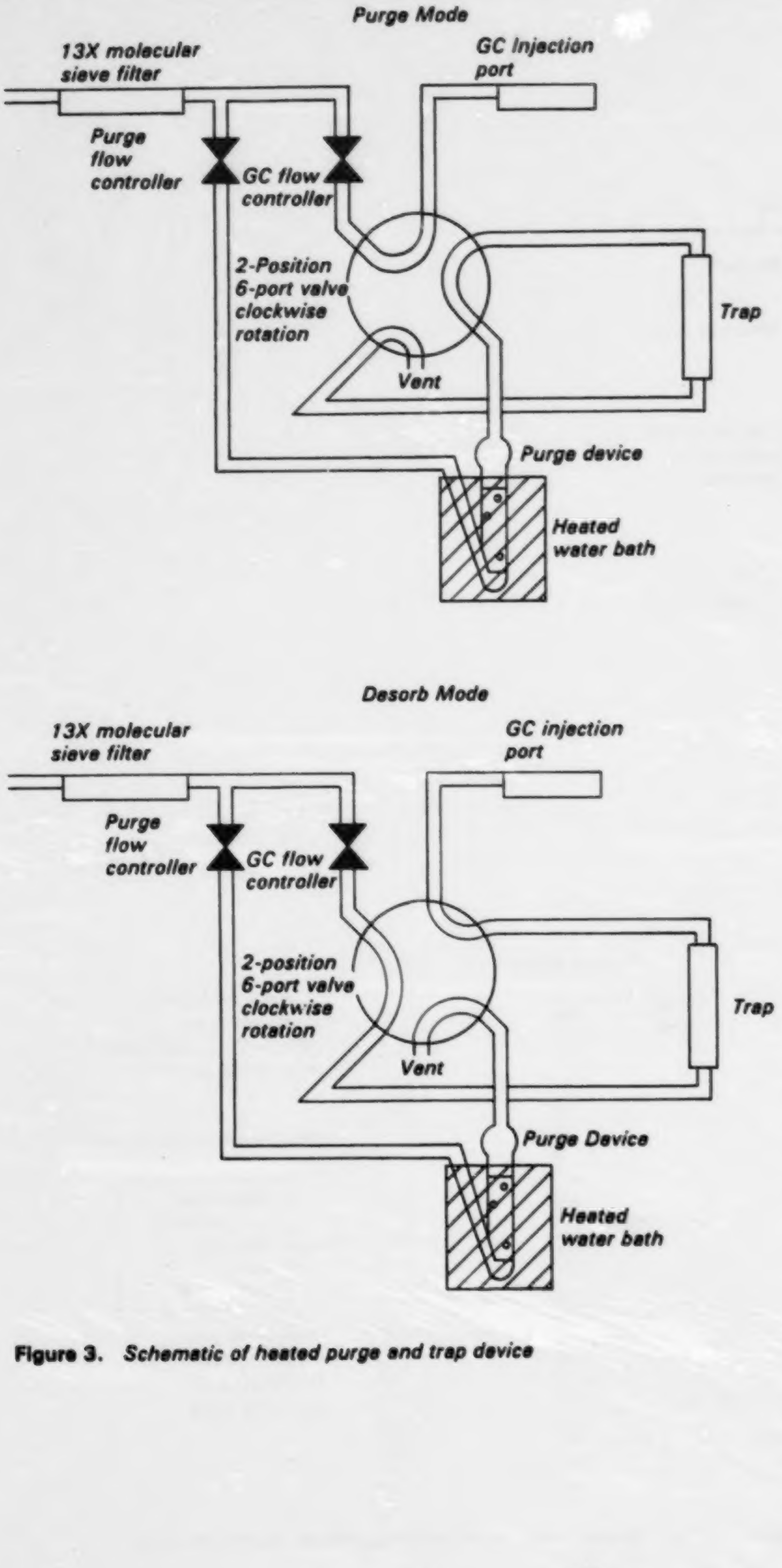

Purge Mode
13X molecular sieve filter
GC Injection port
Purge flow controller
GC flow controller
2-Position 6-port valve clockwise rotation
Trap
Vent
Purge device
Heated water bath
Desorb Mode
13X molecular sieve filter
GC injection port
Purge flow controller
GC flow controller
2-position 6-port valve clockwise rotation
Trap
Vent
Purge Device
Heated water bath

Figure 3. *Schematic of heated purge and trap device*

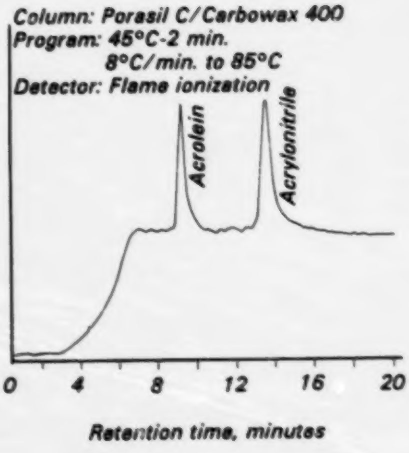


Figure 4. *Gas chromatogram of acrolein and acrylonitrile*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Phenols — Method 604

1. Scope and Application

1.1 This method covers the determination of phenol and certain substituted phenols. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
4-Chloro-3-methylphenol	34452	59-50-7
2-Chlorophenol	34586	95-57-8
2,4-Dichlorophenol	34601	120-83-2
2,4-Dimethylphenol	34606	105-67-9
2,4-Dinitrophenol	34616	51-28-5
2-Methyl-4,6-dinitrophenol	34657	534-52-1
2-Nitrophenol	34591	88-75-5
4-Nitrophenol	34646	100-02-7
Pentachlorophenol	39032	87-86-5
Phenol	34694	108-95-2
2,4,6-Trichlorophenol	34621	88-06-2

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for derivatization, cleanup and electron capture gas chromatography that can be used to confirm measurements made by flame ionization. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL, defined in Section 14.1) (1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix. The MDL listed in Table 1 for each parameter was achieved with a flame ionization detector. Comparable results were achieved when the derivatization cleanup and the electron capture detector were employed (See Table 2).

1.4 Any modification of this method, beyond that expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CRF 136.4 and 136.5.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A 1-liter sample of wastewater is acidified and extracted with methylene chloride using separatory funnel techniques. The extract is dried and concentrated to a volume of 10 mL or less. During the concentration step, the solvent is exchanged to 2-propanol. Flame ionization gas chromatographic conditions are described which allow for the measurement of the compounds in the extract [2].

2.2 A preliminary sample wash under basic conditions can be employed for samples having high general organic and organic base interferences.

2.3 The method also provides for the preparation of pentafluorobenzyl bromide (PFBB) derivatives for electron capture gas chromatography as an additional cleanup procedure to aid in the elimination of interferences [2,3].

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[4]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 12 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the method detection limits listed in Tables 1 and 2.

3.3 The basic sample wash (Section 10.2) may cause significantly reduced recovery of phenol and 2,4-dimethylphenol. The analyst must recognize that results obtained under these conditions are minimum concentrations.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [5-7] for the information of the analyst.

4.2 Special care should be taken in handling pentafluorobenzyl bromide, which is a lachrymator, and 18 crown 6 ether, which is highly toxic.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and capliner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - The sampler must incorporate glass containers for the collection of a minimum of 250 mL of sample. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing must be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2. Drying column - Chromatographic column 400-mm long x 19-mm ID with coarse frit.

5.2.3. Chromatographic column - 100-mm long x 10-mm ID, with Teflon stopcock.

5.2.4. Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.3 Vials - Amber glass, 10- to 15-mL capacity, with Teflon-lined screwcap.

5.4 Reaction flask - Pyrex glass, 15- to 25-mL round bottom flask with standard tapered joint, fitted with a water cooled condenser and U-shaped drying tube containing granular calcium chloride.

5.5 Boiling chips - Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

5.6 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.7 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.8 Gas chromatograph - An analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.8.1 Column for underivatized phenols - 1.8 m long x 2 mm ID glass, packed with 1% SP-1240 DA on Supelcoport (80/100 mesh) or equivalent. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 11.1.

5.8.2 Column for derivatized phenols - 1.8 m long x 2 mm ID glass column packed with 5% OV-17 on Chromosorb W-AW-DMCS (80/100 mesh). The carrier gas is 5% methane/95% Argon at a flow rate of 30 mL/min. The column temperature is 200°C. This column has proven effective in the analysis of wastewaters for derivatization products of the parameters listed in the scope (Section 1.1), and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate columns are provided in Section 11.1.

5.8.3 Detectors - flame ionization and electron capture. The flame ionization is used when determining the parent phenols. The electron capture is used when determining the derivatized phenols. Guidelines for use of alternate detectors are provided in Section 11.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium hydroxide solution (10 N) - (ACS) Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sodium hydroxide solution (1 N) - (ACS) Dissolve 4g NaOH in reagent water and dilute to 100 mL.

6.4 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.

6.5 Sodium thiosulfate - (ACS) Granular.

6.6 Sulfuric acid solution (1+1) - (ACS) Slowly, add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.7 Sulfuric acid (1 N) - (ACS) Slowly, add 29 mL H_2SO_4 (ACS, sp. gr. 1.84) to reagent water and dilute to one liter.

6.8 Potassium carbonate - (ACS) powdered.

6.9 Pentafluorobenzyl bromide (a-Bromopentafluorotoluene) - 97% minimum purity. NOTE: This chemical is a lachrymator. (See Section 4.2.)

6.10 18-crown-6 ether (1,4,7,10,13,16 - Hexaoxacyclooctadecane) - 98% minimum purity. NOTE: This chemical is highly toxic.

6.11 Derivatization reagent - Add one mL pentafluorobenzyl bromide and one gram 18 crown 6 ether to a 50-mL volumetric flask and dilute to volume with 2-propanol. Prepare fresh weekly. This operation should be carried out in a hood. Store 4°C and protect from light.

6.12 Acetone, hexane, methanol, methylene chloride, 2-propanol, hexane, toluene - Pesticide quality or equivalent.

6.13 Silica gel - Davison chemical, grade 923 (100/200 mesh) or equivalent. Activate at 130°C overnight and store in a desiccator.

6.14 Stock standard solutions (1.00 $\mu g/\mu L$) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.14.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in pesticide quality 2-propanol and dilute to volume in a 10-mL volumetric flask. Larger volumes may be prepared at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.14.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268.

6.14.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicates a problem.

7. Calibration

7.1 To calibrate the FIDGC for the analysis of underivatized phenols, establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure for FIDGC.

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with 2-propanol. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure for FIDGC. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with 2-propanol. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1}\quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard, (µg/L).

C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant ($<$ 10% RSD), the RF can be assumed to be nonvariant and the averge RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 To calibrate the ECGC for the analysis of phenol derivatives, establish gas chromatographic operating parameters equivalent to those indicated in Table 2.

7.4.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with 2-propanol. One of the external standards should represent a concentration near but above the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.4.2 Each time samples are to be derivatived, simultaneously treat a one-mL aliquot of each calibration standard as described in Section 12.

7.4.3 After derivatization, inject 2 to 5 µL of each column eluate collected and tabulate peak height or area responses against the calculated equivalent mass of underivatized phenol injected. The results can be used to prepare a calibration curve for each compound.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyse a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in 2-propanol 1000 times more concentrated that the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 3, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X-R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R − 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(8) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± S. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(8).

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(9) should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Fill the sample bottle and at time of collection if residual chlorine is present, add 80 mg of sodium thiosulfate and mix well. U.S. Environmental methods 330.4 and 330.5 may be used for measurement of residual chlorine(10) Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction(2).

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 For samples high inorganic content, the analyst may solvent wash the sample at a basic pH as prescribed in Section 10.2.1 and 10.2.2 to remove potential method interferences. Prolonged or exhaustive contact with solvent during the wash may result in low recovery of some of the phenols, notably phenol and 2,4-dimethyl phenol. For relatively clean samples, the wash should be omitted and the extraction, beginning with Section 10.3, should be followed.

10.2.1 Adjust the pH of the sample to 12.0 or greater with 10 N sodium hydroxide.

10.2.2 Add 60 mL of methylene chloride to the sample by shaking the funnel for one minute with periodic venting to release vapor pressure. Discard to solvent layer. The wash can be repeated up to two additional times if significant color is being removed.

10.3 Adjust the sample to a pH of 1 to 2 with sulfuric acid (1+1).

10.4 Add 60 mL of methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and shake for two minutes. Allow the solvent to separate from the sample and collect the methylene chloride in a 250-mL Erlenmeyer flask. If the emulsion interface between layers is more than one-third the size of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, or centrifugation.

10.5 Add a second 60-mL volume of methylene chloride to the sample bottle and complete the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.6 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the KD if the requirements of Section 8.2 are met.

10.7 Pour the combined extract through a drying column containing three to four inches of anhydrous sodium sulfate, and collect in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL methylene chloride to complete the quantitative transfer.

10.8 Add one to two clean boiling chips to the flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed in vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling.

10.9 Increase the temperature of the hot water bath to 95 to 100°C. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of 2-propanol. A 5-mL syringe is recommended for this operation. Attach a micro-Snyder column to the concentrator tube and prewet the column by adding about 0.5 mL of 2-propanol to the top. Place the micro-K-D apparatus on the water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 minutes. At the proper rate of distillation, the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reached 2.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Add an additional 2 mL of 2-propanol through the top of the micro-Snyder column and resume concentrating as before. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum amount of 2-propanol. Adjust the extract volume to 1.0 mL. Stopper the concentrator tube and store in refrigerator at 4°C, if further processing will not be performed immediately. If the sample extract requires no further cleanup, proceed with flame ionization gas chromatographic analysis (Section 11). If the sample requires further cleanup, proceed to Section 12. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap vials.

10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Gas Chromatography - Flame Ionization Detector

11.1 Table 1 summarizes the recommended gas chromatographic column and operating conditions. This Table includes retention times and MDL obtained under these conditions. An example of the parameter separation achieved by this column is shown in Figure 1. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

11.2 Calibrate the system daily as described in Section 7.1.

11.3 If the internal standard approach is used, the standard must be added to the sample extract and mixed thoroughly immediately before injection into the instrument.

11.4 Inject 2 to 5 μL of the sample extract using the solvent-flush technique[11]. Smaller (1.0 μL) volumes may be injected if automatic injectors are employed. Record the volume injected to the nearest 0.05 μL and the resulting responses in peak area or peak height units. If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

11.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound may be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.6 If the measurement of the peak response is prevented by the presence of interferences, an alternate gas chromatographic procedure is required. Section 12 describes a derivatization and column chromatographic procedure which has been tested and found to be a practical means of analyzing phenols in complex extracts.

12. Derivatization and Electron Capture Gas Chromatography

12.1 Pipet a 1.0-mL aliquot of the 2-propanol solution of standard or sample extract into a glass reaction vial. Add 1.0-mL of derivatizing reagent (Section 6.11). This is a sufficient amount of reagent to derivatize a solution whose total phenolic content does not exceed 0.3 mg/mL.

12.2 Add about 3 mg of potassium carbonate to the solution and shake gently.

12.3 Cap the mixture and heat it for four hours at 80°C in a hot water bath.

12.4 Remove the solution from the hot water bath and allow it to cool.

12.5 Add 10 mL of hexane to the reaction flask and shake vigorously for one minute. Add 3.0 mL of distilled, deionized water to the reaction flask and shake for two minutes. Decant a portion of the organic layer into a concentrator tube and cap with a glass stopper.

12.6 Pack a 10-mm ID chromatographic column with 4.0 grams of activated silica gel. After settling the silica gel by tapping the column, add about two grams of anhydrous sodium sulfate to the top.

12.7 Pre-elute the column with 6 mL of hexane. Discard the eluate and just prior to exposure of the sulfate layer to air, pipet onto the column 2.0 mL of the hexane solution (Section 12.5) that contains the derivatized sample or standard. Elute the column with 10.0 mL of hexane (Fraction 1) and discard this fraction. Elute the column, in order, with: 10.0 mL of 15% toluene in hexane (Fraction 2); 10.0 mL of 40% toluene in hexane (Fraction 3); 10.0 mL 75% toluene in hexane (Fraction 4); and 10.0 mL 15% 2-propanol in toluene (Fraction 5). All elution mixtures are prepared on a volume:volume basis. Elution patterns for the phenolic derivatives are shown in Table 2. Fractions may be combined as desired, depending upon the specific phenols of interest or level of interferences.

12.8 Analyze the fractions by electron capture gas chromatography. Table 2 summarizes the recommended gas chromatographic column and operating conditions. This Table includes retention times and MDL obtained under these conditions. An example of the parameter separation achieved by this column is shown in Figure 2.

12.9 Calibrate the system daily with a minimum of three aliquots of calibration standards, containing each of the phenols of interest that are derivatized according to the procedure (See Section 7.4).

12.10 Inject 2 to 5 μL of the column fractions using the solvent-flush technique. Smaller (1.0 μL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 μL, and the resulting peak size, in area units or height. If the peak response exceeds the linear range of the system, dilute the extract and reanalyze.

13. Calculations

13.1 Calculate the concentration of individual compounds in the sample determined by the flame ionization procedure (without derivatization) as indicated below.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)\,(V_t)}{(V_i)\,(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected (μL).

V_t = Volume of total extract (μL).

V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

$$\text{Eq. 3. Concentration, } \mu g/L = \frac{(A_s)\,(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract (μg).

V_o = Volume of water extracted, in liters.

13.2 Calculate the concentration of individual compounds in the sample when determined by the derivatization and electron capture procedure according to Equation 4:

$$\text{Eq. 4. Concentration, } \mu g/L = \frac{(A)\,(B)\,(V_t)\,(10)}{(V_i)\,(V_s)\,(C)\,(D)}$$

where:

A = Mass of underivatized phenol represented by area of peak in sample chromatogram, determined from calibration curve in Section 7.4.3, in nanograms.

B = Total volume of 2-propanol extract after concentration in mL.

V_i = Volume of eluate injected (μg/L).

V_t = Total volume of column eluate (μg/L).

V_s = Volume of water extracted (mL).

C = Volume of hexane sample solution added to cleanup column, in mL.

D = Volume of 2-propanol extract used for derivatization in mL.

13.3 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.4 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in 8.4, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Tables 1 and 2 were obtained using reagent water(12). Similar results were achieved using representative wastewaters.

14.2 In a single laboratory (I.T. Enviroscience, Inc.), using spiked reagent water and wastewater samples, the average recoveries presented in Table 3 were obtained using a flame ionization detector(12). The standard deviation of the percent recovery is also included in Table 3.

14.3 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. "Determination of Phenols in Industrial and Municipal Wastewaters," Report for EPA Contract 68-03-2625. In preparation.
3. Kawahara, F.K., *Analytical Chemistry, 40,* 1009 (1968).
4. ASTM Annual Book of Standard, Part 31, D3694, "Standards Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
5. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
6. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
7. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
8. "Handbook for Analytical Quality Control in Wastewater and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
9. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
10. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
11. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).
12. "Development of Detection Limits, EPA Method 604, Phenols." Special letter report for EPA contract 68-03-2625. Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limit*

Parameter	Retention Time (min.)	Method Detection Limit (µg/L)
2-Chlorophenol	1.70	0.31
2-Nitrophenol	2.00	0.45
Phenol	3.01	0.14
2,4-Dimethylphenol	4.03	0.32
2,4-Dichlorophenol	4.30	0.39
2,4,6-Trichlorophenol	6.05	0.64
4-Chloro-3-methylphenol	7.50	0.36
2,4-Dinitrophenol	10.00	13.0
2-Methyl-4,6-dinitrophenol	10.24	16.0
Pentachlorophenol	12.42	7.4
4-Nitrophenol	24.25	2.8

Column conditions: Supelcoport (80/100 mesh) coated with 1% SP-1240 DA in 1.8 m long x 2 mm ID glass column with nitrogen carrier gas at a flow rate of 30 mL/min flow rate. Column temperature was 80°C at injection, programmed immediately at 8°C/min to 150°C final temperature. Method detection limits were determined with a flame ionization detector.

Table 2. *Silica Gel Fractionation and Electron Capture Gas Chromatography of PFBB Derivatives*

	Recovery (%) by Fraction[a]					Electron Capture	
Parent Compound	1	2	3	4	5	Retention Time (min.)	Method Detection Limit (µg/L)
2-Chlorophenol	-	-	90	1	-	3.3	0.58
2-Nitrophenol	-	-	-	9	90	9.1	0.77
Phenol	-	-	90	10	-	1.8	2.2
2,4-Dimethylphenol	-	-	95	7	-	2.9	0.63
2,4-Dichlorophenol	-	-	95	1	-	5.8	0.68
2,4,6-Trichlorophenol	-	50	50	-	-	7.0	0.58
4-Chloro-2-methyphenol	-	-	84	14	-	4.8	1.8
Pentachlorophenol	-	75	20	-	-	28.8	0.59
4-Nitrophenol	-	-	-	1	90	14.0	0.70

[a] *Eluting solvent compositions as given in Section 12.7.*

Column conditions: Chromosorb W-AW-DMCS (80/100 mesh) coated with 5% OV-17 packed in a 1.8 m long x 2.0 mm ID glass column with 5% methane/95% argon carrier gas at a flow rate of 30 mL/min. Column temperature isothermal at 200°C.

Table 3. *Single Operator Accuracy and Precision*

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
4-Chloro-3-methylphenol	82	15.0	0.70 - 3.5	21	3
2-Chlorophenol	67	14.8	0.74 - 3.7	21	3
2,4-Dichlorophenol	74	11.4	1.03 - 5.2	21	3
2,4-Dimethylphenol	51	14.0	0.82 - 4.1	21	3
2,4-Dinitrophenol	74	16.5	28.7	14	2
2-Methyl-4,6-dinitrophenol	86	12.4	34.6	21	3
2-Nitrophenol	67	12.9	0.80 - 4.0	21	3
4-Nitrophenol	45	7.9	15.9	21	3
Pentachlorophenol	79	8.8	21.0	21	3
Phenol	41	8.4	0.76 - 3.8	21	3
2,4,6-Trichlorophenol	71	14.5	1.20 - 6.0	21	3

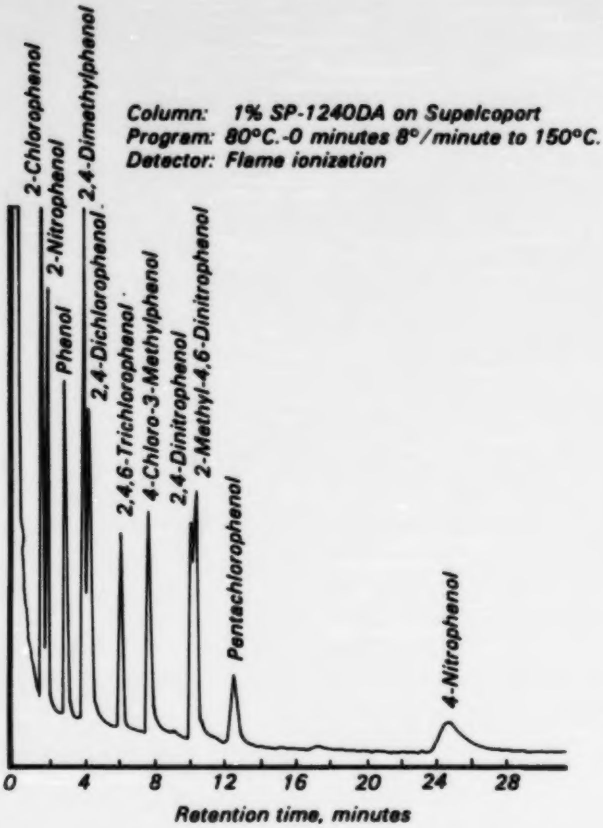


Figure 1. *Gas chromatogram of phenols*

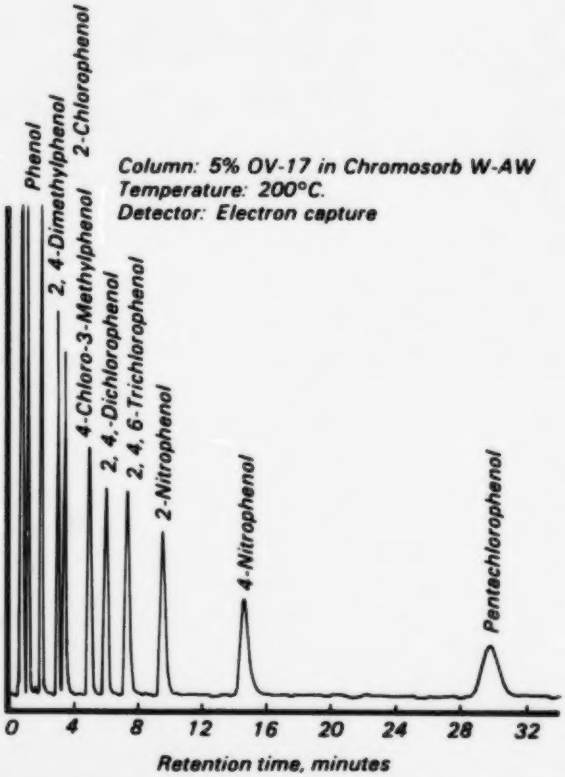


Figure 2. *Gas chromatogram of PFB derivatives of phenols.*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Benzidines — Method 605

1. Scope and Application

1.1 This method covers the determination of certain benzidines. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Benzidine	39120	92-87-5
3,3'-Dichlorobenzidine	34631	91-94-1

1.2 This is a high performance liquid chromatography (HPLC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for the compounds above, identifications should be supported by, at least, one additional qualitative technique. This method decribes electrochemical conditions at a second potential which can be used to confirm measurements made with this method. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL defined in Section 14)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ depending upon the nature of the interferences in the sample matrix.

1.4 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC instrumentation and in the interpretation of liquid chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is extracted with chloroform using liquid-liquid extractions in a separatory funnel. The chloroform extract is back-extracted with acid, neutralized, and extracted with chloroform. The chloroform is exchanged to methanol and concentrated using a rotary evaporator and nitrogen blowdown. It is then brought to a volume of 5 mL with an acetate buffer. HPLC conditions are described which permit the separation and measurement of the benzidine compounds using an electrochemical detector (2).

2.2 The acid back extraction step acts as a general purpose cleanup to aid in the elimination of interferences.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned(3). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and reagent water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures that are inherent in the extraction step are used to overcome many of these interferences, however unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

3.3 Some dye plant effluents contain large amounts of components with retention times close to benzidine. In these cases, it has been found useful to reduce the electrode potential in order to eliminate interferences yet still detect benzidine. (See Section 12.7.)

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and are identified(4-6) for the benefit of the analyst.

4.2 Benzidine, 3,3'-dichlorobenzidine and chloroform, used for extraction of samples have been tentatively classified as known or suspected, human or mammalian carcinogens.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone, or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested.)

5.2.1 Separatory funnels - 2000, 1000, and 250-mL, with Teflon stopcock.

5.2.2 Vials - Amber glass, 10- to 15-mL capacity, with Teflon-lined screw cap.

5.2.3 Rotary evaporator.

5.2.4 Flasks, round bottom, 100-mL, with 24/40 joints.

5.2.5 Centrifuge tubes - conical, screw-capped, graduated, equipped with Teflon lined caps.

5.2.6 Pipettes - Pasteur, with bulbs.

5.3 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.4 HPLC - An analytical system complete with column supplies, compatible recorder, high pressure syringes and the following components.

5.4.1 Solvent delivery system - with pulse damper, Altex 110A, or equivalent.

5.4.2 Injection valve (Optional) - Waters U6K or equivalent.

5.4.3 Electrochemical detector - Bioanalytical Systems LC-2A with glassy carbon electrode, or equivalent. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope, and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1.

5.4.4 Electrode polishing kit - Princeton Applied Research Model 9320 or equivalent.

5.4.5 Column - Lichrosorb RP-2, 5 micron particle diameter, in a 25 cm x 4.6 mm ID stainless steel column. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium hydroxide solution (5 N) - (ACS) Dissolve 20g NaOH in reagent water and dilute to 100 mL.

6.3 Sodium hydroxide (1 M)-(ACS) - Dissolve 40 g NaOH in reagent water and dilute to one liter.

6.4 Sodium thiosulfate - (ACS) Granular.

6.5 Sodium tribasic phosphate (0.4 M) - (ACS) Dissolve 160 g of trisodium phosphate decahydrate in reagent water and dilute to one liter.

6.6 Sulfuric acid solution (1+1) - (ACS) Slowly, add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.7 Sulfuric acid (1 M) - (ACS) Slowly add 58 mL H_2SO_4 (sp. gr. 1.84) to reagent water and dilute to one liter.

6.8 Acetate buffer (0.1 M, pH-4.7) Dissolve 5.8 mL glacial acetic acid (ACS) and 13.6 g of sodium acetate trihydrate (ACS) in reagent water which has been purified by filtration through a RO-4 Millipore System or equivalent and dilute to one liter.

6.9 Acetonitrile, chloroform (preserved with 1% ethanol), methanol - Pesticide quality or equivalent.

6.10 Mobile phase - Place equal volumes of filtered acetonitrile (Millipore type FH filter or equivalent) and filtered acetate buffer (millipore type GS filter or equivalent) in a narrow-mouth glass container and mix thoroughly. Prepare fresh weekly. Degas daily by sonicating under vacuum, or by heating and stirring, or by purging with helium.

6.11 Stock standard solutions (1.00 $\mu g/\mu L$) - Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions.

6.11.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality methanol and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.11.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check samples that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.11.3 Stock standard solutions must be replaced after six months, or sooner if comparison with quality control check samples indicate a problem.

7. Calibration

7.1 Establish chromatographic operating parameters equivalent to those given in Table 1. The HPLC system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with mobile phase. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using syringe injections of 5 to 25 μL or a constant volume injection loop, inject each calibration standard, and tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound. If serious loss of sensitivity occurs, polish the electrode and recalibrate.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with mobile phase. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using 5 to 25 μL aliquots or a constant volume injection loop, inject each calibration standard, and tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard, ($\mu g/L$).

C_s = Concentration of the parameter to be measured, ($\mu g/L$).

If the RF value over the working range is a constant (< 10% RSD), the RF can be assumed to be nonvariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound. If serious loss of response occurs, polish the electrode and recalibrate.

7.4 Before using any alternate cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X-R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = $R + 3s$

Lower Control Limit (LCL) = $R - 3s$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(7) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(7).

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as HPLC with a dissimilar column, gas chromatography, or mass spectrometry must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(8) should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C and stored in the dark from the time of collection until extraction. Both benzidine and 3,3'-dichlorobenzidine are easily oxidized. Fill the sample bottle and, at time of collection, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample, and mix thoroughly. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used for measurement of residual chlorine[9]. Field test kits are available for this purpose. After mixing, adjust the pH of the sample to a range of 2 to 7 with sulfuric acid.

9.3 If 1,2-diphenyl hydrazine is likely to be present, adjust the pH of the sample to 4.0 ± 0.2 to prevent rearrangement to benzidine.

9.4 All samples must be extracted within seven days. Extracts may be held up to seven days before analysis, if stored under an inert (oxidant free) atmosphere[2]. The extract should be protected from light.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 6.5 to 7.5 with sodium hydroxide or sulfuric acid solutions.

10.2 Add 100 mL chloroform to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. (Caution: Handle chloroform in a well ventilated area.) Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the chloroform extract in a 250-mL separatory funnel.

10.3 Add a 50-mL volume of chloroform to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the separatory funnel. Perform a third extraction in the same manner.

10.4 Separate and discard any aqueous layer remaining in the 250-mL separatory funnel after combining the organic extracts. Add 25 mL of 1 M sulfuric acid and extract the sample by shaking the funnel for two minutes. Transfer the aqueous layer to a 250-mL beaker. Extract with two additional 25-mL portions of 1 M sulfuric acid and combine the acid extracts in the beaker.

10.5 Place a stirbar in the 250-mL beaker and stir the acid extract while carefully adding 5 mL of 0.4 M sodium tribasic phosphate. While monitoring with a pH meter, neutralize the extract to a pH between 6 and 7 by dropwise addition of 5 N NaOH while stirring the solution vigorously. Approximately 25 to 30 mL of 5 N NaOH will be required and it should be added over at least a two-minute period. Do not allow the sample pH to exceed 8.

10.6 Transfer the neutralized extract into a 250-mL separatory funnel. Add 30 mL of chloroform and shake the funnel for two minutes. Allow the phases to separate, and transfer the organic layer to a second 250-mL separatory funnel.

10.7 Extract the aqueous layer with two additional 20-mL aliquots of chloroform as before. Combine the extracts in the 250-mL separatory funnel.

10.8 Add 20 mL of reagent water to the combined organic layers and shake for 30 seconds.

10.9 Transfer the organic extract into a 100-mL round bottom flask. Add 20 mL of methanol and concentrate to 5 mL with a rotary evaporator at reduced pressure and 35°C. An aspirator is recommended for use as the source of vacuum. Chill the receiver with ice. This operation requires approximately 10 minutes. Other concentration techniques may be employed, if the requirements of Section 8.2 are met.

10.10 Using a 9-inch Pasteur pipette, transfer the extract to a 15 mL conical screw-capped centrifuge tube. Rinse the flask, including the entire side wall, with 2-mL portions of methanol and combine with the original extract.

10.11 Carefully concentrate the extract to 0.5 mL using a gentle stream of nitrogen while heating in a 30°C water bath. Dilute to 2 mL with methanol, reconcentrate to 1 mL, and dilute to 5 mL with acetate buffer. Mix extract thoroughly. Cap the centrifuge tube and store refrigerated and protected from light if further processing will not be performed immediately.

10.12 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Additional cleanup procedures may not be necessary for a relatively clean sample matrix. The single operator precision and accuracy data in Section 14 were gathered using only those cleanup procedures that are inherent in the extraction procedures of this method. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

12. Liquid Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the HPLC. This Table includes retention times, capacity factors, and MDL that were obtained under these conditions. An example of the parameter separation achieved by the HPLC column is shown in Figure 1. Other HPLC columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. When the HPLC is idle, it is advisable to maintain a 0.1 mL/min flow through the column to prolong column life.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, the standard must be added to the sample extract, and mixed thoroughly immediately, before injection into the instrument.

12.4 Inject 5 to 25 µL of the sample extract. If constant volume injection loops are not used, record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract with mobile phase and reanalyze.

12.7 If the measurement of the peak response for benzidine is prevented by the presence of interferences, reduce the electrode potential to +0.6 volts and reanalyze. If the benzidine peak is still obscured by interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from the equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected (μL).

V_t = Volume of total extract (μL).

V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

$$\text{Eq. 3. Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract (μg).

V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.4, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(10). Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of analyte recovery from reagent water and has been demonstrated to be applicable over the concentration range from 7 x MDL to 3000 x MDL(10).

14.3 In a single laboratory (Battelle, Columbus Laboratories), using spiked wastewater samples, the average recoveries presented in Table 2 were obtained(2). Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the percent recovery is also included in Table 2.

14.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. "Determination of Benzidines in Industrial and Municipal Wastewaters," Report for EPA Contract 68-03-2624 (In preparation).
3. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
4. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
5. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
7. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
8. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
9. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.
10. "Determination of Method Detection Limit and Analytical Curve for EPA Method 605 - Benzidines," Special letter report for EPA Contract 68-03-2624, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	Retention Time (min)	Column Capacity Factor	Method Detection Limit (µg/L)
Benzidine	6.1	1.44	0.08
3,3'-dichlorobenzidine	12.1	3.84	0.13

HPLC Column conditions: Lichrosorb RP-2, 5 micron particle size, in a 25 cm x 4.6 mm ID stainless steel column. Mobile Phase: 0.8 mL/min of 50% acetonitrile/50% 0.1M pH 4.7 acetate buffer. The method detection limit was determined using an electrochemical detector operated at + 0.8 volts.

Table 2. *Single Operator Accuracy and Precision*

Parameter	Average Percent Recovery	Standard Deviation %	Spike range (µg/L)	Number of Analyses	Matrix Types
Benzidine	65	11.4	1.0-50	30	5
3,3'-Dichlorobenzidine	64	9.6	1.0-50	30	5

Column: Lichrosorb RP-2
Mobile phase: 50% Acetonitrile in acetate buffer
Detector: Electrochemical at +0.8 volts

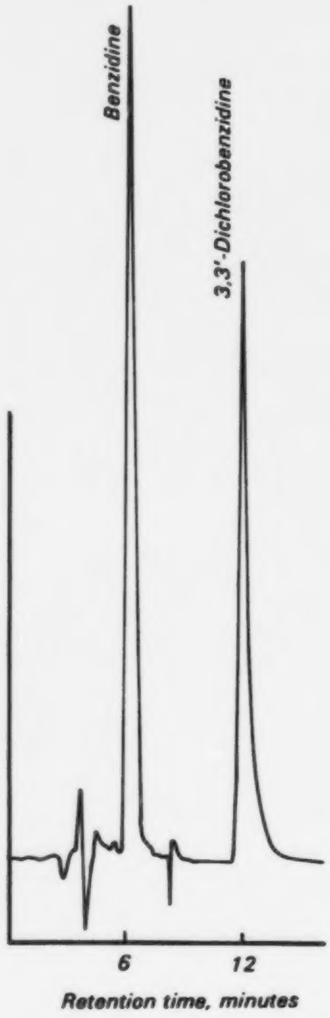


Figure 1. *Liquid chromatogram of benzidines*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Phthalate Esters — Method 606

1. Scope and Application

1.1 This method covers the determination of certain phthalate esters. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Bis(2-ethylhexyl) phthalate	39100	117-81-7
Butyl benzyl phthalate	34292	85-68-7
Di-n-butyl phthalate	39110	84-74-2
Diethyl phthalate	34336	84-66-2
Dimethyl phthalate	34341	131-11-3
Di-n-octyl phthalate	34596	117-84-0

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL, defined in Section 14)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in methods 608, 609, 611 and 612. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary to apply appropriate cleanup procedures. The analyst is allowed the latitude, under Gas Chromatography (Section 12), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CRF 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately 1-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract by electron capture gas chromatography[2].

2.2 Analysis for phthalates is especially complicated by their ubiquitous occurrence in the environment. This method provides Florisil and alumina column cleanup procedures to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[3]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Phthalate esters are contaminants in many products commonly found in the laboratory. It is particularly important to avoid the use of plastics because phthalates are commonly used as plasticizers and are easily extracted from plastic materials. Serious phthalate contamination can result at any time, if consistent quality control is not practiced. Great care must be experienced to prevent such contamination. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination[4,5].

3.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified [6-8] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container and cap liner must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse frit.

5.2.3 Chromatographic column - 300 mm long x 10 mm ID with coarse fritted disc at bottom and Teflon stopcock (Kontes K-420540-0213 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.8 Vials - Amber glass, 10- to 15-mL capacity, with Teflon-lined screw-cap.

5.3 Boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.6 Gas chromatograph - Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 180 cm long x 4 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 12.1.

5.6.2 Column 2 - 180 cm long x 4 mm ID glass, packed with 3% OV-1 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector - Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Acetone, hexane, isooctane, methylene chloride, methanol - Pesticide quality or equivalent.

6.3 Ethyl ether - Nanograde, redistilled in glass if necessary.

6.3.1 Must be free of peroxides as indicated by EM Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8 and others.)

6.3.2 Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.4 Sodium sulfate - (ACS) Granular, anhydrous. Several levels of purification may be required in order to reduce background phthalate levels to an acceptable level: 1) Heat four hours at 400°C in a shallow tray, 2) Heat 16 hours at 450-500°C in a shallow tray, 3) Soxhlet extract with methylene chloride for 48 hours.

6.5 Florisil - PR grade (60/100 mesh). Purchase activated at 1250°F and store in dark in glass container with ground glass stopper or foil-lined screw cap. To prepare for use, place 100g of Florisil into a 500-mL beaker and heat for approximately 16 hours at 400°C. After heating transfer to a 500-mL reagent bottle. Tightly seal and cool to room temperature. When cool add 3 mL of reagent water. Mix thoroughly by shaking or rolling for 10 minutes and let it stand for at least two hours. Keep the bottle sealed tightly.

6.6 Alumina - Neutral activity Super I, W200 series, (ICN Life Sciences Group, No. 404583). To prepare for use, place 100 g of alumina into a 500-mL beaker and heat for approximately 16 hours at 400°C. After heating transfer to a 500-mL reagent bottle. Tightly seal and cool to room temperature. When cool add 3 mL of reagent water. Mix thoroughly by shaking or rolling for 10 minutes and let it stand for at least two hours. Keep the bottle sealed tightly.

6.7 Stock standard solutions (1.00 μg/μL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in pesticide quality isooctane, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be at a concentration near, but above, MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each parameter. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3. The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1 } RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard, (μg/L).
C_s = Concentration of the parameter to be measured, (μg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If s > 2p or |X-R| > 2p, review potential problem areas and repeat the test.

8.2.5 The U. S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R - 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(9) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(9).

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices (10) should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction(2).

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for a least 10 minutes.

10.7 Increase the temperature of the hot water bath to about 80°C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip and reattach the Snyder column. Pour about 1 mL of hexane into the top of the Snyder column and concentrate the solvent extract as before. Elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane and adjust the volume to 10 mL. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup, proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 If the entire extract is to be cleaned up by one of the following two procedures, it must be concentrated to about 2 mL. To the concentrator tube in Section 10.8, add a clean boiling chip and attach a two-ball micro-Snyder column. Prewet the column by adding about 0.5 mL hexane to the top. Place the K-D apparatus on a hot water bath (80°C) so that the concentrator tube is partially immersed in hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with 0.2 mL of hexane. Proceed with one of the following cleanup procedures. Proper use of either procedure should yield acceptable results.

11.3 Florisil column cleanup for phthalate esters

11.3.1 Place 10g of Florisil into a 10 mm ID chromatography column and tap the column to settle the Florisil. Add 1 cm of anhydrous sodium sulfate to the top of the Florisil.

11.3.3 Preelute the column with 40 mL of hexane. Discard this eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 mL sample extract onto the column, using an additional 2 mL of hexane to complete the transfer.

11.3.4 Just prior to exposure of the sodium sulfate layer to the air, add 40 mL hexane and continue the elution of the column. Discard this hexane eluate.

11.3.5 Next elute the phthalate esters with 100 mL of 20% ethyl ether/80% hexane (V/V) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Elute the column at a rate of about 2 mL/min. for all fractions. Concentrate the collected fraction by standard K-D technique. No solvent exchange is necessary. After concentration and cooling, adjust the volume of the cleaned up extract to 10 mL in the concentrator tube and analyze by gas chromatography.

11.4 Alumina column cleanup for phthalate esters.

11.4.1 Place 10g of alumina into a 10 mm ID chromatography column and tap the column to settle the alumina. Add 1 cm of anhydrous sodium sulfate to the top of the alumina.

11.4.3 Preelute the column with 40 mL of hexane. Discard this eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 mL sample extract onto the column, using an additional 2 mL of hexane to complete the transfer.

11.4.4 Just prior to exposure of the sodium sulfate layer to the air add 35 mL of hexane and continue the elution of the column. Discard this hexane eluate.

11.4.5 Next elute the column with 140 mL of 20% ethyl ether/80% hexane (V/V) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Elute the column at a rate of about 2 mL/min. for all fractions. Concentrate the collected fraction by standard K-D technique. No solvent exchange is necessary. After concentration and cooling, adjust the volume of the cleaned up extract to 10 mL in the concentrator tube and analyze by gas chromatography.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. Included in this table are estimated retention times and MDL that can be achieved by this method. Examples of the separations achieved by column 1 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 2 to 5 µL of the sample extract using the solvent-flush technique(11). Smaller (1.0 µL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)\,(V_t)}{(V_i)\,(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected (µL).

V_t = Volume of total extract (µL).

V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

$$\text{Eq. 3. Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract (µg).

V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(12). Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 5 X MDL to 1000 X MDL with the following exceptions: dimethyl and diethyl phthalate recoveries at 1000 X MDL were low (70%); bis-2-ethylhexyl and di-n-octyl phthalate recoveries at 5 X MDL were low (60%)(12).

14.3 In a single laboratory (Southwest Research Institute), using spiked wastewater samples, the average recoveries presented in Table 2 were obtained. Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the percent recovery is also included in Table 2(2).

14.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.

2. "Determination of Phthalates in Industrial and Municipal Wastewaters." Report for EPA Contract 68-03-2606 (In preparation).

3. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

4. Giam, C.S., Chan, H.S. and Neff, G.S., "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples," *Analytical Chemistry, 47,* 2225, (1975).

5. Giam, C.S., Chan, H.S., "Control of Blanks in the Analysis of Phthalates in Air and Ocean Biota Samples," National Bureau of Standards (U.S.), Special Publication 442, pp. 701-708, 1976.

6. "Carcinogens - Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

7. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

8. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

9. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268, March 1979.

10. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

11. Burke, J.A., "Gas Chromatography For Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).

12. "Method Detection Limit and Analytical Curve Studies, EPA Methods 606, 607, and 608." Special letter report for EPA Contract 68-03-2606. Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268.

Table 1. Chromatographic Conditions and Method Detection Limits

Parameter	*Retention Time (min.) Column 1*	*Column 2*	*Method Detection Limit (µg/L)*
Dimethyl phthalate	*2.03*	*0.95*	*0.29*
Diethyl phthalate	*2.82*	*1.27*	*0.49*
Di-n-butyl phthalate	*8.65*	*3.50*	*0.36*
Butyl benzyl phthalate	*6.94**	*5.11**	*0.34*
Bis(2-ethylhexyl) phthalate	*8.92**	*10.5 **	*2.0*
Di-n-octyl phthalate	*16.2 **	*18.0 **	*3.0*

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 packed in a 1.8 m long x 4 mm ID glass column with 5% methane/95% argon carrier gas at a flow rate of 60 mL/min. Column temperature, isothermal at 180°C, except where otherwise indicated.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-1 in a 1.8 m long x 4 mm ID glass column with 5% methane/95% argon carrier gas at 60 mL/min flow rate. Column temperature isothermal 200°C except where otherwise indicated.

**Retention time based upon isothermal oven temperature of 220°C.*

Table 2. Single Operator Accuracy and Precision

Parameter	*Average Percent Recovery*	*Standard Deviation %*	*Spike Range (µg/L)*	*Number of Analyses*	*Matrix Types*
Bis(2-ethylhexyl) phthalate	*85*	*4.2*	*24-1000*	*24*	*4*
Butyl benzyl phthalate	*82*	*6.5*	*3-100*	*24*	*4*
Di-n-butyl phthalate	*80*	*6.2*	*20-1500*	*24*	*4*
Diethyl phthalate	*94*	*1.3*	*15-50*	*18*	*3*
Dimethyl phthalate	*94*	*3.4*	*15-50*	*18*	*3*
Di-n-octyl phthalate	*86*	*4.9*	*40-150*	*24*	*4*

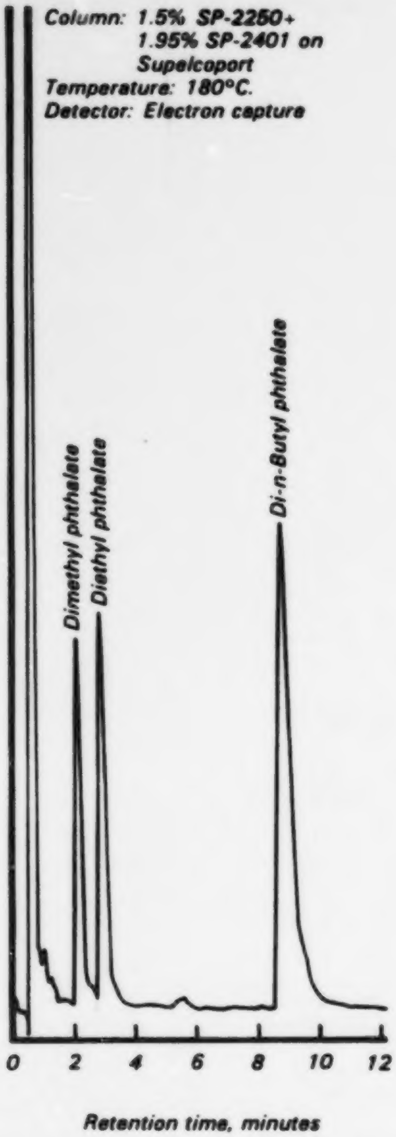


Figure 1. *Gas chromatogram of phthalates.*

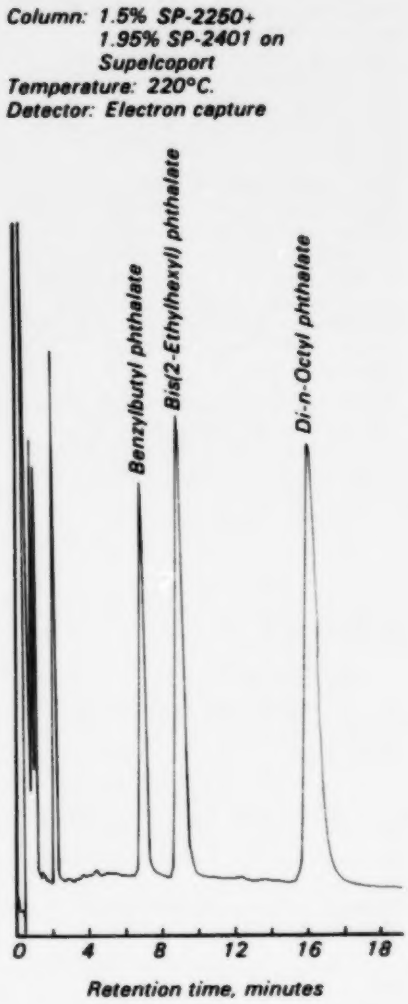


Figure 2. *Gas chromatogram of phthelates.*

63<

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Nitrosamines — Method 607

1. Scope and Application

1.1 This method covers the determination of certain nitrosamines. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
N-Nitrosodimethylamine	34438	62-75-9
N-Nitrosodiphenylamine	34433	86-30-6
N-Nitrosodi-n-propylamine	34428	621-64-7

1.2 This is a gas chromatographic (GC) method applicable to the determination of the parameters listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for N-nitrosodi-n-propylamine. In order to confirm the presence of N-nitrosodiphyenylamine, the cleanup procedure specified in Section 11.3 or 11.4 must be used. In order to confirm the presence of N-nitrosodimethylamine by GC/MS, chromatographic column 1 of this method must be substituted for the column recommended in method 625. Confirmation of these parameters using GC-high resolution mass spectrometry or a Thermal Energy Analyzer is also recommended practice(1,2).

1.3 The method detection limit (MDL defined in Section 14.1)(3) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is washed with dilute HCl to remove free amines, dried and concentrated to a volume of

10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the compounds in the extract after it has been exchanged to methanol.(4)

2.2 The method provides Florisil and alumina column cleanup procedures to separate diphenylamine from the nitrosamines and to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned(5). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400 °C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures (Section 11) can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

3.3 N-Nitrosodiphenylamine is reported(6-9) to undergo transnitrosation reactions. Care must be exercised in the heating or concentrating of solutions containing this compound in the presence of reactive amines.

3.4 The sensitive and selective Thermal Energy Analyzer and the reductive Hall detector may be used in place of the nitrogen-phosphorus detector when interferences are encountered. The Thermal Energy Analyzer offers the highest selectivity of the non-mass spectrometric detectors.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined, however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(10-12) for the information of the analyst.

4.2 These nitrosamines are known carcinogens(13-17), therefore, utmost care must be exercised in the handling of these materials. Nitrosamine reference standards and standard solutions should be handled and prepared in a ventilated glove box within a properly ventilated room.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4 °C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel—2000-mL and 250-mL, with Teflon stopcock.

5.2.2 Drying column—Chromatographic column approximately 400 mm long × 19 mm ID with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish—three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish—two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials—Amber glass, 10- to 15-mL capacity, with Teflon-lined screw cap.

5.2.8 Chromatographic column—Pyrex, approximately 400 mm long × 22 mm ID, with coarse fritted disc at bottom and Teflon stopcock (Kontes K-420540-0234 or equivalent), for use in Florisil column cleanup procedure.

5.2.9 Chromatographic column—Pyrex, approximately 300 mm long × 10 mm ID, with coarse fritted disc at bottom and Teflon stopcock (Kontes K-420540-0213 or equivalent), for use in alumina column cleanup procedure.

5.3 Boiling chips—approximately 10/40 mesh. Heat to 400 °C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (± 2 °C). The bath should be used in a hood.

5.5 Balance—Analytical, capable of accurately weighing 0.0001 g.

5.6 Gas chromatograph—An analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1—1.8 m long × 4 mm ID Pyrex glass, packed with Chromosorb W AW (80/100 mesh) coated with 10% Carbowax 20 M/2% KOH or equivalent. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 12.2.

5.6.2 Column 2—1.8 m long × 4 mm ID, Pyrex glass packed with Supelcoport (100/120 mesh) coated with 10% SP-2250.

5.6.3 Detector—Nitrogen-Phosphorus, reductive Hall or Thermal Energy Analyzer[1,2]. These detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope. A nitrogen-phosphorus detector was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.2.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium hydroxide solution (10 N)—(ACS) Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sodium thiosulfate—(ACS) Granular.

6.4 Sulfuric acid solution (1 + 1)—(ACS) Slowly, add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.5 Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400 °C for 4 hours in a shallow tray.

6.6 Hydrochloric acid (1 + 9)—(ACS) Add one volume of conc. HCl to nine volumes of reagent water.

6.7 Acetone, methanol, methylene chloride and pentane—Pesticide quality or equivalent.

6.8 Ethyl ether—Nanograde, redistilled in glass if necessary.

6.8.1 Must be free of peroxides as indicated by EM Quant test strips. (Test strips are available from Scientific Products Co., Catalog No. P1126-8 and others.)

6.8.2 Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.9 Florisil—PR grade (60/100 mesh); purchase activated at 1250 °F and store in dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch at least 16 hours at 130 °C in a foil covered glass container.

6.10 Alumina—Basic activity Super I, W 200 series (ICN Life Sciences Group, No. 404571). Place 100 g of alumina, as it comes from the manufacturer, into a 500-mL reagent bottle and add 2 mL of reagent water. Mix the alumina preparation thoroughly by shaking or rolling for 10 minutes and let it stand for at least two hours. The preparation should be homogenous before use. Keep the bottle sealed tightly to ensure proper activity.

6.11 Stock standard solutions (1.00 μg/μL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.11.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in pesticide quality methanol, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.11.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4 °C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.11.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Table 1. The gas chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with methanol. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with methanol. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

Eq. 1. $RF = (A_s C_{is})/(A_{is} C_s)$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard, (µg/L).

C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be nonvariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X - R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = $R + 3s$
Lower Control Limit (LCL) = $R - 3s$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(18) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(18).

8.4. The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[19] should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4 °C from the time of collection until extraction. Fill the sample bottle and at time of collection, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used for measurement of residual chlorine[20]. Field test kits are available for this purpose. If diphenylnitrosamine is to be determined, adjust the sample pH to 7 to 10 with sodium hydroxide or sulfuric acid.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction[4].

9.4 Nitrosamines are known to be light sensitive[7]. Samples should be stored in amber or foil-wrapped bottles in order to minimize photolytic decomposition.

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5 to 9 with sodium hydroxide or sulfuric acid.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Add 10 mL of HCl (1 + 1) solution to the combined extracts and shake for two minutes. Allow the layers to separate. Transfer the combined extract to a drying column containing 10 cm of anhydrous sodium sulfate and collect it in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles.

10.7 If N-nitrosodiphenylamine is to be measured by gas chromatography, the analyst must first use a cleanup column to eliminate diphenylamine interference (Section 11). If N-nitrosodiphenylamine is of no interest, the analyst may proceed directly with gas chromatographic analysis (Section 12).

10.8 Determine the original sample volume by refilling the sample bottle to the mark with water and measure it in a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%. Diphenylamine, if present in the original sample extract must be separate from the nitrosamines if N-nitrosodiphenylamine is to be determined by this method.

11.2 If the entire extract is to be cleaned up by one of the following procedures, it must be concentrated to 2.0 mL. To the concentrator tube in Section 10.6, add a clean boiling chip and attach a two-ball micro-Snyder column. Prewet the column by adding about 0.5 mL of methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apparatus from the hot water bath and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with 0.2 mL of methylene chloride. Adjust the final volume to 2.0 mL and proceed with one of the following cleanup procedures.

11.3 Florisil column cleanup for nitrosamines:

11.3.1 Place 22 g of activated Florisil in a 22 mm ID chromatographic column. After settling the Florisil by tapping the column, add about a 5-mm layer of anhydrous granular sodium sulfate to the top.

11.3.2 Preelute the column, after colling, with 40 mL of ethyl ether/pentane (15 + 85)(V/V). Discard the eluate and just prior to exposure of the sodium sulfate layer to air, quantitatively transfer a 2.0 mL aliquot of the sample extract into the column by decantation using an additional 2 mL of pentane to complete the transfer.

11.3.3 Perform the first elution with 90 mL of ethyl ether/pentane (15 + 85)(V/V) and discard the eluate. This fraction will contain the diphenylamine, if it is present in the extract.

11.3.4 Perform the second elution with 100 mL of acetone/ethyl ether (5 + 95)(V/V) and collect the eluate in a 500-mL K-D flask equipped with a 10-mL concentrator tube. This fraction will contain all of the nitrosamines listed in the scope of the method.

11.3.5 Add 15 mL of methanol to the collected eluate and concentrate as in Section 10.6 at 70 to 75 °C, using pentane for the diluting and rinsing solvent.

11.3.6 Analyze by gas chromatography.

11.4 Alumina column cleanup for nitrosamines:

11.4.1 Place 12 g of the alumina preparation into a 10 mm ID chromatographic column and tap the column to settle the alumina. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the alumina.

11.4.2 Preelute the column with 10 mL of ethyl ether/pentane (3 + 7)(V/V). Discard the eluate (about 2 mL) and, just prior to exposure of the sodium sulfate layer to air, transfer a 2.0 mL aliquot of the sample extract onto the column by decantation using an additional 2 mL of pentane to complete the transfer.

11.4.3 Just prior to exposure of the sodium sulfate layer to the air, add 70 mL of ethyl ether/pentane (3 + 7)(V/V). Discard the first 10 mL of eluate. Collect the remainder of the eluate in a 500-mL K-D flask equipped with a 10-mL concentrator tube. This fraction contains N-nitrosodiphenylamine and probably a small amount of N-nitrosodi-n-propylamine.

11.4.4 Next elute the column with 60 mL of ethyl ether/pentane (1 + 1) (V/V), collecting the eluate in a second K-D flask equipped with a 10-mL concentrator tube. Add 15 mL of methyl alcohol to the K-D flask. This fraction will contain N-nitrosodimethylamine, most of the N-nitrosodi-n-propylamine and any diphenylamine that is present.

11.4.5 Concentrate both fractions as in Section 10.6 using pentane as the diluting and rinsing solvent.

11.4.6 Analyze the fractions by gas chromatography.

12. Gas Chromatography

12.1 N-nitrosodiphenylamine completely reacts to form diphenylamine at the normal operating temperatures of a GC injection port (200 to 250 °C). Thus, N-nitrosodiphenylamine is chromatographed and detected as diphenylamine. Accurate determination depends on removal of diphenylamine that may be present in the original extract prior to GC (See Section 11).

12.2 Table 1 summarizes the recommended operating conditions for the gas chromatograph. This Table includes retention times and MDL that were obtained under these conditions. Examples of the parameter separations achieved by these columns are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.3 Calibrate the system daily as described in Section 7.

12.4 If the extract has not been submitted to one of the cleanup procedures in Section 11, it is necessary to exchange the solvent from methylene chloride to methyl alcohol before the thermionic detector can be used. To a 1-10 mL volume of methylene chloride extract in a concentrator tube, add 2 mL methyl alcohol, and a clean boiling chip. Attach a two-ball micro-Snyder column. Prewet the column by adding about 0.5 mL methylene chloride through the top. Place the K-D apparatus on a boiling water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position and insulate the apparatus as necessary to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with 0.2 mL of methyl alcohol. Adjust the final volume to 2.0 mL.

12.5 If the internal standard approach is being used, add the internal standard to sample the extract and mix thoroughly, immediately before injection into the instrument.

12.6 Inject 2 to 5 µL of the sample extract using the solvent-flush technique[21]. Smaller (1.0 µL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.7 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.8 If the response exceeds the working range of the system, dilute the extract and reanalyze.

12.9 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected (μL).

V_t = Volume of total extract (μL).

V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

Eq. 3

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract (μg).

V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(3). The MDL concentrations listed in Table 1 were obtained using reagent water(22). Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of analyte recovery from reagent water and has been demonstrated to be applicable over the concentration range from 4 × MDL to 1000 × MDL(22).

14.3 In a single laboratory (Southwest Research Institute), using spiked wastewater samples, the average recoveries presented in Table 2 were obtained(4). Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the percent recovery is also included in Table 2.

14.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. Fine, D.H., Lieb, D. and Rufeh, R., "Principle of Operation of the Thermal Energy Analyzer for the Trace Analysis of Volatile and Non-volatile N-nitroso Compounds." *Journal of Chromatography, 107,* 351, (1975).

2. Fine, D.H. Hoffman, F., Rounbehler, D.P. and Belcher, N.M., "Analysis of N-nitroso Compounds by Combined High Performance Liquid Chromatography and Thermal Energy Analysis," Walker, E.A., Bogovski, P. and Griciute, L., editors, N-Nitroso Compounds—Analysis and Formation Lyon, International Agency for Research on Cancer (IARC Scientific Publications No. 14), pp. 43-50, (1976)

3. See Appendix A.

4. "Determination of Nitrosamines in Industrial and Municipal Wastewaters." Report for EPA Contract 68-03-2606 (In preparation).

5. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

6. Buglass, A.J., Challis, B.C. and Osborn, M.R. "Transnitrosation and Decomposition of Nitrosamines." Bogovski, P. and Walker, E.A., editors, N-Nitroso Compounds in the Environment, Lyon, International Agency for Research on Cancer (IARC Scientific Publication No. 9), pp. 94-100, (1974).

7. Burgess, E.M. and Lavanish, J.M., "Photochemical Decomposition of N-nitrosamines," *Tetrahedon Letters,* 1221, (1964).

8. Druckrey, H., Preussmann, R., Ivankovic, S. and Schmahl, D., "Organotrope Carcionogene Wirkungen bei 65 Verschiedenen N-Nitroso-Verbindungen an BD-Ratten." *Z. Krebsforsch., 69,* 103 (1967).

9. Fiddler, W. "The Occurrence and Determination of N-nitroso Compounds," *Toxicol. Appl. Pharmacol., 31,* 352, (1975).

10. "Carcinogens—Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

11. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

12. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

13. Lijinsky, W., "How Nitrosamines Cause Cancer." *New Scientist, 73,* 216, (1977)

14. Mirvish, S.S. "N-Nitroso compounds: Their Chemical and in vivo Formation and Possible Importance as Environmental Carcinogens." *J. Toxicol. Environ. Health, 3,* 1267 (1977).

15. "Reconnaissance of Environmental Levels of Nitrosamines in the Central United States." National Enforcement Investigations Center, Environmental Protection Agency, Report No. EPA-330/1-77-001, (1977)

16. "Atmospheric Nitrosamine Assessment Report." Office of Air Quality Planning and Standards, Environmental Protection Agency, Research Triangle Park, North Carolina, (1976).

17. "Scientific and Technical Assessment Report on Nitrosamines." Office of Research and Development, Environmental Protection Agency, Report No. EPA-660/6-7-001, (1976).

18. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

19. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.

20. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600-4/79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

21. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).

22. "Method Detection Limit and Analytical Curve Studies EPA Methods 606, 607, 608," special letter report for EPA Contract 68-03-2606. Environmental Monitoring and Support Laboratory—Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	*Retention Time (min.)* *Column 1*	 *Column 2*	*Method Detection Limit μg/L*
N-Nitrosodimethylamine	*4.1*	*0.88*	*0.15*
N-Nitrosodi-n-propylamine	*12.1*	*4.2*	*0.46*
N-Nitrosodiphenylamine[a]	*12.8*[b]	*6.4*[c]	*0.81*

Column 1 conditions: Chromosorb W AW (80/100 mesh) coated with 10% Carbowax 20 M/2% KOH packed in a 1.8 m long × 4 mm ID glass column with helium carrier gas at a flow rate of 40 mL/min. column temperature Isothermal, at 110°C, except as otherwise indicated.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 10% SP-2250 packed in a 1.8 m long × 4 mm ID glass column with helium carrier gas at a flow rate of 40 mL/min. column temperature, Isothermal at 120°C, except as otherwise indicated.

[a] *Measured as diphenylamine*

[b] *Determined isothermally at 220°C.*

[c] *Determined isothermally at 210°C.*

Table 2. *Single Operator Accuracy and Precision*

Parameter	*Average Percent Recovery*	*Standard Deviation %*	*Spike Range (μg/L)*	*Number of Analyses*	*Matrix Types*
N-Nitrosodimethylamine	*32*	*3.7*	*0.8*	*29*	*5*
N-Nitrosodiphenylamine	*79*	*7.1*	*1.2*	*29*	*5*
N-Nitrosodi-n-propylamine	*61*	*4.2*	*9.0*	*29*	*5*

Column: 10% Carbowax 20M + 2%
KOH on Chromosorb W-AW
Temperature: 110°
Detector: Phosphorus/Nitrogen

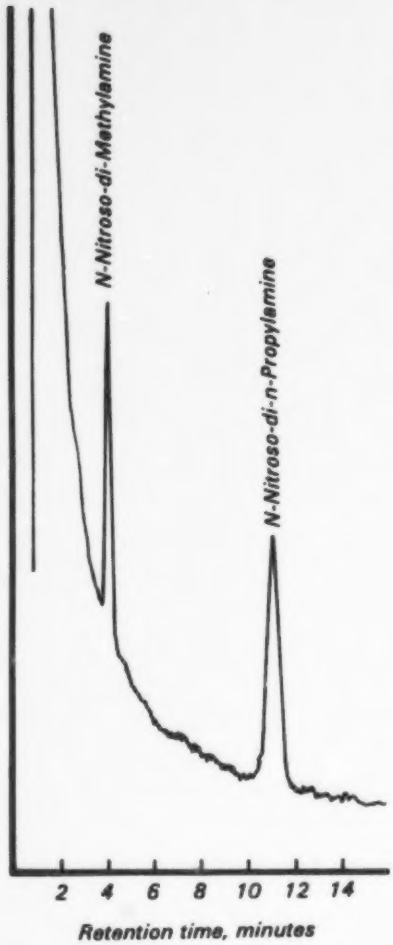


Figure 1. Gas chromatogram of nitrosamines.

Column: 10% Carbowax 20M + 2% KOH on
Chromosorb W-AW
Temperature: 220°C.
Detector: Phosphorus/Nitrogen

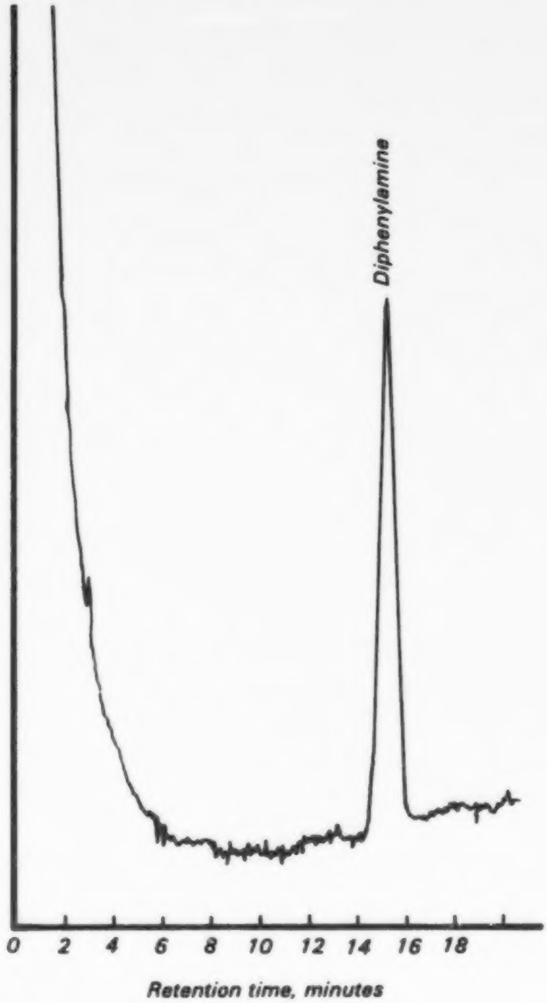


Figure 2. Gas chromatogram of N-nitrosodiphenylamine as diphenylamine.

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Organochlorine Pesticides and PCBs — Method 608

1. Scope and Application

1.1 This method covers the determination of certain organochlorine pesticides and PCBs. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Aldrin	39330	309-00-2
α-BHC	39337	319-84-6
β-BHC	39338	319-85-7
δ-BHC	34259	319-86-8
γ-BHC	39340	58-89-9
Chlordane	39350	57-74-9
4,4′-DDD	39310	72-54-8
4,4′-DDE	39320	72-55-9
4,4′-DDT	39300	50-29-3
Dieldrin	39380	60-57-1
Endosulfan I	34361	959-98-8
Endosulfan II	34356	33212-65-9
Endosulfan sulfate	34351	1031-07-8
Endrin	39390	72-20-8
Endrin aldehyde	34366	7421-93-4
Heptachlor	39410	76-44-8
Heptachlor epoxide	39420	1024-57-3
Toxaphene	39400	8001-35-2
PCB-1016	34671	12674-11-2
PCB-1221	39488	11104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL, defined in Section 14.1)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in methods 606, 609, 611 and 612. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selection of aliquots as necessary to apply appropriate cleanup procedures. The analyst is allowed the latitude to select gas chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane, during concentration to a final volume of 10 mL or less. Gas chromatographic conditions are described which permit the separation and measurement of the parameters in the extract by electron capture GC(2).

2.2 The method provides a Florisil column procedure and elemental sulfur removal procedure to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned(3). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry and heated in a muffle furnace at 400 °C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually elminates PCB interference. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Interferences by phthalate esters can pose a major problem in pesticide analysis when using the elution capture detector. These compounds generally appear in the chromatogram as large eluting peaks, especially in the 15 and 50% fractions from Florisil. Common flexible plastics contain varying amounts of phthalates. These phthalates are easily extracted or leached from such materials during laboratory operations. Cross contamination of clean glassware routinely occurs when plastics are handled during extraction steps, especially when solvent wetted surfaces are handled. Interferences from phthalates can best be minimized by avoiding the use of plastics in the laboratory. Exhaustive cleanup of reagents and glassware may be required to eliminate background phthalate contamination(4,5). The interferences from phthalate esters can be avoided by using a microcoulometric or electrolytic conductivity detector.

3.3 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(6-8) for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: 4,4′-DDT,4,4′-DDD, the BHCs, and the PCBs. Primary standards of these toxic compounds should be prepared in a hood.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4 °C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel—2000-mL, with Teflon stopcock.

5.2.2 Drying column—Chromatographic column approximately 400 mm long × 19 mm ID, with coarse frit.

5.2.3 Chromatographic column—Pyrex, 400 mm long × 22 mm ID, with coarse fritted plate and Teflon stopcock (Kontes K-42054 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish—three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials—Amber glass, 10- to 15-mL capacity, with Teflon-lined screw cap.

5.3 Boiling chips—approximately 10/40 mesh. Heat to 400 °C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (± 2 °C). The bath should be used in a hood.

5.5 Balance—Analytical, capable of accurately weighing 0.0001 g.

5.6 Gas chromatograph—An analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1—1.8 m long × 4 mm ID glass, packed with 1.5% SP-2250/1.95% SP-2401 on Supelcoport (100/120 mesh) or equivalent. Column 1 was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 12.1.

5.6.2 Column 2—1.8 m long × 4 mm ID glass, packed with 3% OV-1 on Supelcoport (100/120 mesh) or equivalent.

5.6.3 Detector—Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope, and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium hydroxide solution (10 N)—(ACS). Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sodium thiosulfate—(ACS). Granular.

6.4 Sulfuric acid solution (1 + 1)—(ACS). Slowly, add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.5 Acetone, hexane, isooctane (2,2,4-trimethylpentane), methylene chloride—Pesticide quality or equivalent.

6.6 Ethyl ether—Pesticide quality or equivalent, redistilled in glass if necessary.

6.6.1 Must be free of peroxides as indicated by EM Laboratories Quant test strips (Available from Scientific Products Co., Cat. No. P1126-8, and others suppliers.)

6.6.2 Procedures recommended for removal of peroxides are provided with the test strips. After cleanup, 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.7 Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400 °C for 4 hours in a shallow tray.

6.8 Florisil—PR grade (60/100 mesh); purchase activated at 1250 °F and store in dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch at least 16 hours at 130 °C in a foil covered glass container.

6.9 Mercury—Triple distilled.

6.10 Copper powder—Activated.

6.11 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.11.1 Prepare stock standard solutions by accurately weighing about 0.0100 grams of pure material. Dissolve the material in isooctane, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.11.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4 °C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.11.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters which produce retention times equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

Eq. 1. $RF = (A_sC_{is})/(A_{is}C_s)$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard, (μg/L).

C_s = Concentration of the parameter to be measured, (μg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 The cleanup procedure in Section 11 utilizes Florisil chromatography. Florisil from different batches or sources may vary in absorptive capacity. To standardize the amount of Florisil which is used, the use of lauric acid value[9] is suggested. The referenced procedure determines the adsorption from hexane solution of lauric acid (mg) per gram Florisil. The amount of Florisil to be used for each column is calculated by dividing this factor into 110 and multiplying by 20 g.

7.5 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X - R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (LCL) = R − 3s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(10) that are useful in observing trends in performance. The control limits above be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(10).

8.4. The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.5. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(11) should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4 °C from the time of collection until extraction. If the samples will not be extracted within 72 hours of collection, the sample should be adjusted to a pH range of 5.0 to 9.0 with sodium hydroxide or sulfuric acid. Record the volume of acid or base used. If aldrin is to be determined, add sodium thiosulfate when residual chlorine is present. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used to measure chlorine residual(12). Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction(2).

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the Kuderna Danish if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

10.7 Increase the temperature of the hot water bath to about 80 °C. Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip and reattach the Snyder column. Prewet the column by adding about 1 mL of hexane to the top. Concentrate the solvent extract as before. The elapsed time of concentration should be 5 to 10 minutes. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool at least 10 minutes.

10.8 Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles. If the sample extract requires no further cleanup, proceed with gas chromatographic analysis. If the sample requires cleanup proceed to Section 11.

10.9 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%. The Florisil column allows for a select fractionation of the compounds and will eliminate polar materials. Elemental sulfur interferes with the electron capture gas chromatography of certain pesticides, but can be removed by the techniques described below.

11.2 Florisil column cleanup:

11.2.1 Add a weight of Florisil (nominally 21 g) predetermined by calibration (Section 7.4 and 7.5), to a chromatographic column. Settle the Florisil by tapping the column. Add sodium sulfate to the top of the Florisil to form a layer 1 to 2 cm deep. Add 60 mL of hexane to wet and rinse the sodium sulfate and Florisil. Just prior to exposure of the sodium sulfate to air, stop the elution of the hexane by closing the stopcock on the chromatography column. Discard the eluate.

11.2.2 Adjust the sample extract volume to 10 mL with hexane and transfer it from the K-D concentrator tube to the Florisil column. Rinse the tube twice with 1 to 2 mL hexane, adding each rinse to the column.

11.2.3 Place a 500-mL K-D flask and clean concentrator tube under the chromatography column. Drain the column into the flask until the sodium sulfate latyer is nearly exposed. Elute the column with 200 mL of 6% ethyl ether in hexane (V/V) (Fraction 1) using a drip rate of about 5 mL/min. Remove the K-D flask and set aside for later concentration. Elute the column again, using 200 mL of 15% ethyl ether in hexane (V/V)(Fraction 2), into a second K-D flask. Perform the third elution using 200 mL of 50% ethyl ether in hexane (V/V)(Fraction 3). The elution patterns for the pesticides an PCB's are shown in Table 2.

11.2.4 Concentrate the eluates by standard K-D techniques (Section 10.6), substituting hexane for the glassware rinses and using the water bath at about 85 °C. Adjust final volume to 10 mL with hexane. Analyze by gas chromatography.

11.3 Elemental sulfur will usually elute entirely in Fraction 1 of the Florisil column cleanup. To remove sulfur interference from this fraction or the original extract, pipet 1.00 mL of the concentrated extract into a clean concentrator tube or Teflon-sealed vial. Add one to three drops of mercury and seal(13). Agitate the contents of the vial for 15 to 30 seconds. Prolonged shaking (two hours) may be required. If so, this may be accomplished with a reciprocal shaker. Alternatively, activated copper powder may be used for sulfur removal(14). Analyze by gas chromatography.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. This table includes retention times and MDL that were obtained under these conditions. Examples of the parameter separations achieved by column 1 are shown in Figures 1 to 10. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, the internal standard must be added to the sample extract and mixed thoroughly immediately, before injection into the instrument.

12.4 Inject 2 to 5 µL of the sample extract using the solvent-flush technique(15). Smaller (1.0 µL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, the total extract volume, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected (µL).

V_t = Volume of total extract (µL).

V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

Eq. 3

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

I_s = Amount of internal standard added to each extract (µg).

V_o = Volume of water extracted, in liters.

13.2 When it is apparent that two or more PCB (Aroclor) mixtures are present, the Webb and McCall procedure(16) may be used to identify and quantify the Aroclors.

13.3 For multicomponent mixtures (chlordane, toxaphene and PCBs) match retention times of peaks in the standards with peaks in the sample. Quantitate every identifiable peak unless interference with individual peaks persist after cleanup. Add peak height or peak area of each identified peak in the chromatogram. Calculate as total response in the sample versus total response in the standard.

13.4 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.5 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(17). Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of spike recovery from reagent water and has been demonstrated to be applicable over the concentration range from 4 × MDL up to 1000 × MDL with the following exceptions: Chlordane recovery at 4 × MDL was low (60%); Toxaphene recovery was demonstrated linear over the range of 10 × MDL to 1000 × MDL(17).

14.3 In a single laboratory (Southwest Research Institute), using spiked wastewater samples, the average recoveries presented in Table 3 were obtained(4). Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the percent recovery is also included in Table 3.

14.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. "Determination of Pesticides and PCBs in Industrial and Municipal Wastewaters." Report for EPA Contract 68-03-2606. In preparation.
3. ASTM Annual Book of Standards, Part 31, D3694, "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
4. Giam, D.S., Chan, H.S. and Nef, G.S., "Sensitive Method for Determination of Phthalate Ester Plasticizers in Open-Ocean Biota Samples," *Analytical Chemistry, 47,* 2225, (1975).
5. Giam, C.S., Chan, H.S., "Control of Blanks in the Analysis of Phthalates in Air and Ocean Biota Samples," U.S. National Bureau of Standards, Special Publication 442, pp. 701-708, 1976.
6. "Carcinogens—Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
7. "OSHA Safety and Health Standards, General Industry," (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
8. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
9. Mills, P.A., "Variation of Florisil Activity: Simple Method for Measuring Absorbent Capacity and Its Use in Standardizing Florisil Columns," *Journal of the Association of Official Analytical Chemists, 51,* 29 (1968).
10. "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
11. ASTM Annual Book of Standards, Part 31, D3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA. p. 76, 1980.
12. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600-4/79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
13. Goerlitz, D.F. and Law, L.M., *Bulletin for Environmental Contamination and Toxicology, 6* 9 (1971).
14. "Manual of Analytical Methods for the Analysis of Pesticides in Human Environmental Samples," U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, N.C., EPA Report 600/8-80-038, Section 11,B, p.6.
15. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).
16. Webb, R.G., and McCall, A.C., "Quantitative PCB Standards for Electron Capture Gas Chromatography," *Journal of Chromatographic Science, 11,* 366 (1973).
17. "Method Detection Limit and Analytical Curve Studies, EPA Methods 606, 607, and 608," Special letter report for EPA Contract 68-03-2606. Environmental Monitoring and Support Laboratory—Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	*Retention Time (min.)* Column 1	*Retention Time (min.)* Column 2	*Method Detection Limit μg/L*
α-BHC	1.35	1.82	0.003
γ-BHC	.70	2.13	0.004
β-BHC	1.90	1.97	0.006
Heptachlor	2.00	3.35	0.003
δ-BHC	2.15	2.20	0.009
Aldrin	2.40	4.10	0.004
Hepachlor epoxide	3.50	5.00	0.083
Endosulfan I	4.50	6.20	0.014
4,4'-DDE	5.13	7.15	0.004
Dieldrin	5.45	7.23	0.002
Endrin	6.55	8.10	0.006
4,4'-DDD	7.83	9.08	0.011
Endosulfan II	8.00	8.28	0.004
4,4'-DDT	9.40	11.75	0.012
Endrin aldehyde	11.82	9.30	0.023
Endosulfan sulfate	14.22	10.70	0.066
Chlordane	mr	mr	0.014
Toxaphene	mr	mr	0.24
PCB-1016	mr	mr	nd
PCB-1221	mr	mr	nd
PCB-1232	mr	mr	nd
PCB-1242	mr	mr	0.065
PCB-1248	mr	mr	nd
PCB-1254	mr	mr	nd
PCB-1260	mr	mr	nd

Column 1 conditions: Supelcoport (100/120 mesh) coated with 1.5% SP-2250/1.95% SP-2401 packed in a 1.8 m long × 4 mm ID glass column with 5% Methane/95% Argon carrier gas at a flow rate of 60 mL/min. Column temperature isothermal at 200°C, except for PCB-1016 through PCB-1248, which should be measured at 160°C.

Column 2 conditions: Supelcoport (100/120 mesh) coated with 3% OV-1 in a 1.8 m long × 4 mm ID glass column with 5% Methane/95% Argon carrier gas at a flow rate of 60 mL/min. Column temperature, isothermal at 200°C, for the pesticides; 140°C for PCB-1221 and 1232; 170°C for PCB-1016 and 1242 to 1268.

mr — Multiple peak response. See Figures 2 thru 10.

nd — Not determined.

Table 2. *Distribution of Chlorinated Pesticides and PCBs into Florisil Column Fractions*[2]

Parameter	*Percent Recovery by Fraction* Fraction 1	*Percent Recovery by Fraction* Fraction 2	*Percent Recovery by Fraction* Fraction 3
Aldrin	100		
α-BHC	100		
β-BHC	97		
δ-BHC	98		
γ-BHC	100		
Chlordane	100		
4,4'-DDD	99		
4,4'-DDE	98		
4,4'-DDT	100		
Dieldrin	0	100	
Endosulfan I	37	64	
Endosulfan	0	7	91
Endosulfan sulfate	0	0	106
Endrin	4	96	
Endrin aldehyde	0	68	26
Heptachlor	100		
Heptachlor epoxide	100		
Toxaphene	96		
PCB-1016	97		
PCB-1221	97		
PCB-1232	95	4	
PCB-1242	97		
PCB-1248	103		
PCB-1254	90		
PCB-1260	95		

Eluant composition by fraction:
Fraction 1 — 6% ethyl ether in hexane
Fraction 2 — 15% ethyl ether in hexane
Fraction 3 — 50% ethyl ether in hexane

Table 3. Single Operator Accuracy and Precision

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (μg/L)	Number of Analyses	Matrix Types
Aldrin	89	2.5	2.0	15	3
α-BHC	89	2.0	1.0	15	3
β-BHC	88	1.3	2.0	15	3
δ-BHC	86	3.4	2.0	15	3
γ-BHC	97	3.3	1.0	15	3
Chlorane	93	4.1	20	21	4
4-4'-DDD	92	1.9	6.0	15	3
4,4'-DDE	89	2.2	3.0	15	3
4,4'-DDT	92	3.2	8.0	15	3
Dieldrin	95	2.8	3.0	15	2
Endosulfan I	96	2.9	3.0	12	2
Endosulfan II	97	2.4	5.0	14	3
Endosulfan sulfate	99	4.1	15	15	3
Endrin	95	2.1	5.0	12	2
Endrin aldehyde	87	2.1	12	11	2
Heptachlor	88	3.3	1.0	12	2
Heptachlor epoxide	93	1.4	2.0	15	3
Toxaphene	95	3.8	200	18	3
PCB-1016	94	1.8	25	12	2
PCB-1221	96	4.2	55-110	12	2
PCB-1232	88	2.4	110	12	2
PCB-1242	92	2.0	28-56	12	2
PCB-1248	90	1.6	40	12	2
PCB-1254	92	3.3	40	18	3
PCB-1260	91	5.5	80	18	3

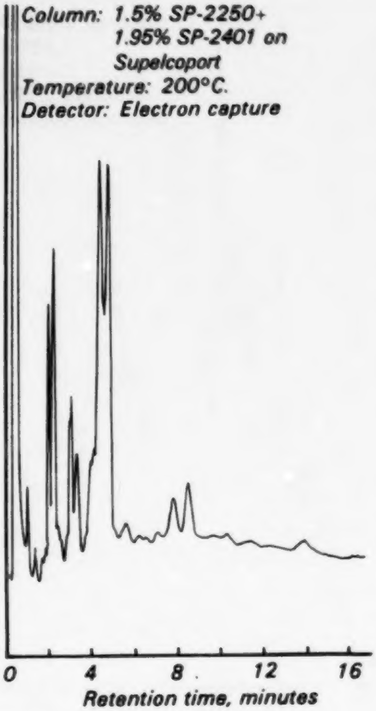


Figure 2. *Gas chromatogram of chlordane.*

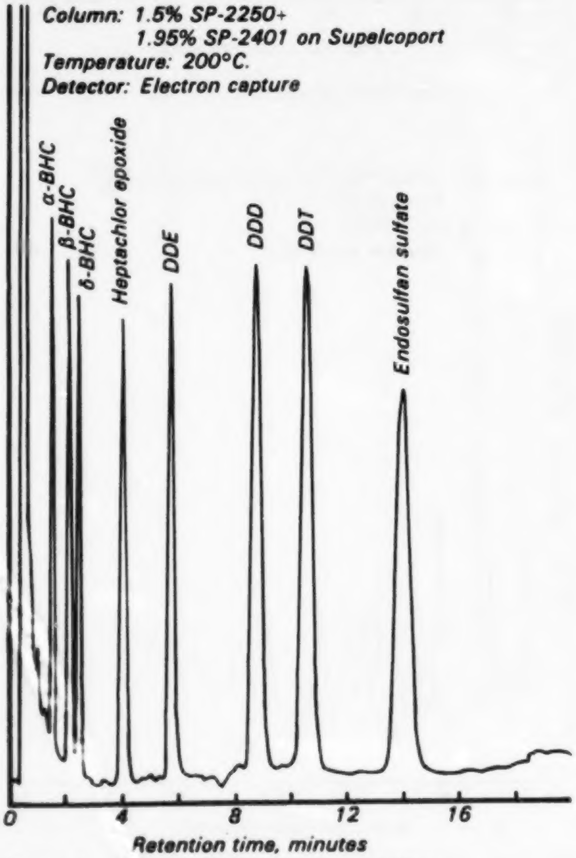


Figure 1. *Gas chromatogram of pesticides.*

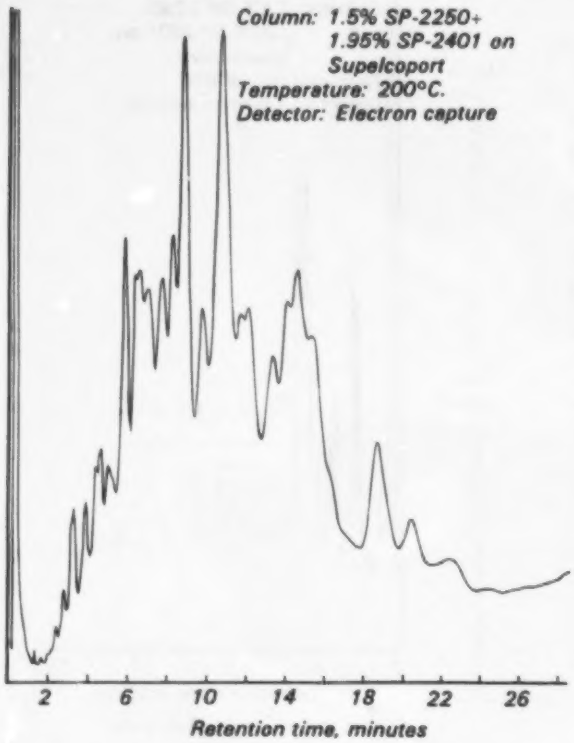


Figure 3. *Gas chromatogram of toxaphene.*

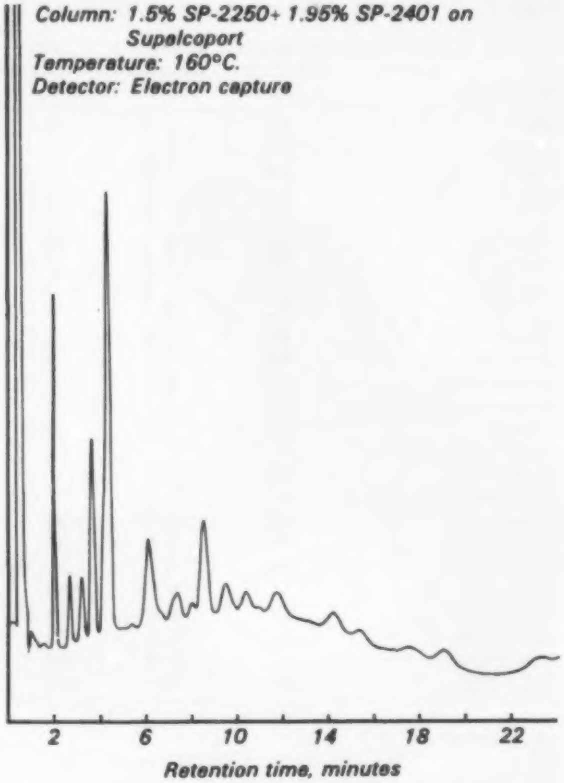


Figure 5. *Gas chromatogram of PCB-1221.*

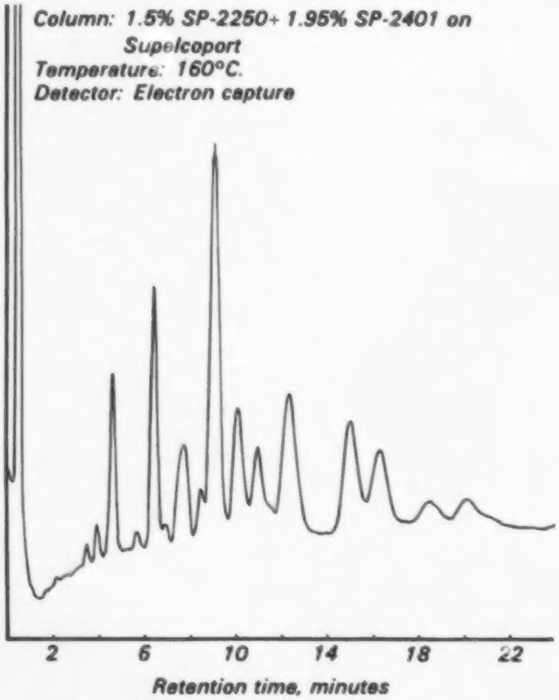


Figure 4. *Gas chromatogram of PCB-1016.*

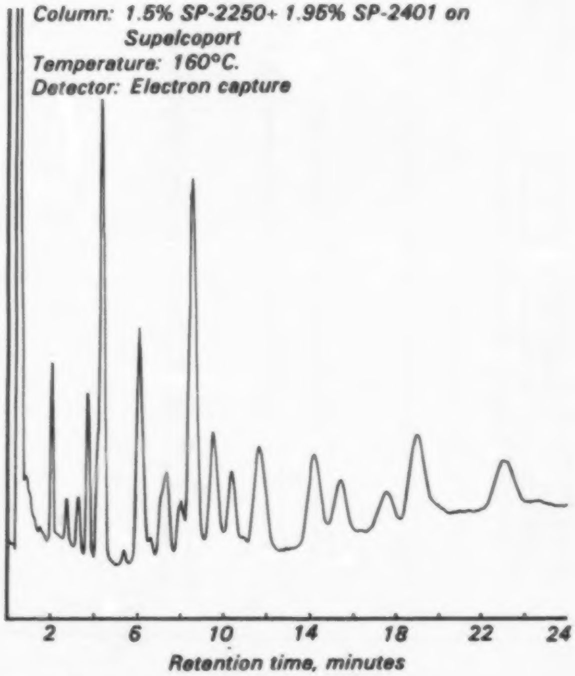


Figure 6. *Gas chromatogram of PCB-1232.*

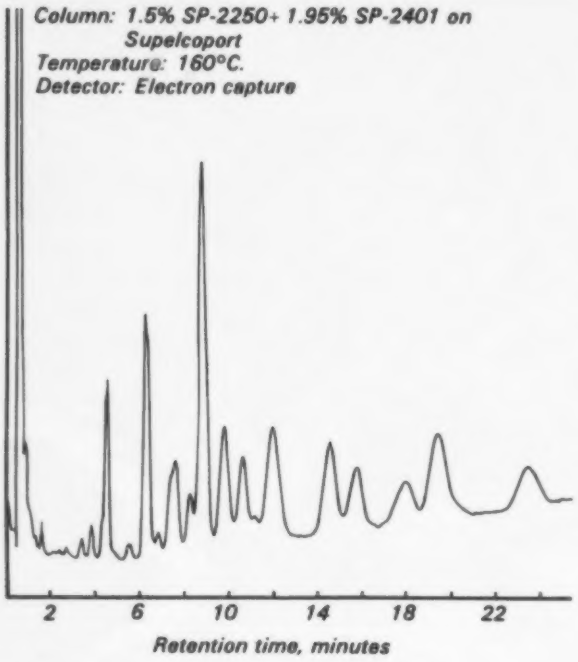


Figure 7. Gas chromatogram of PCB-1242.

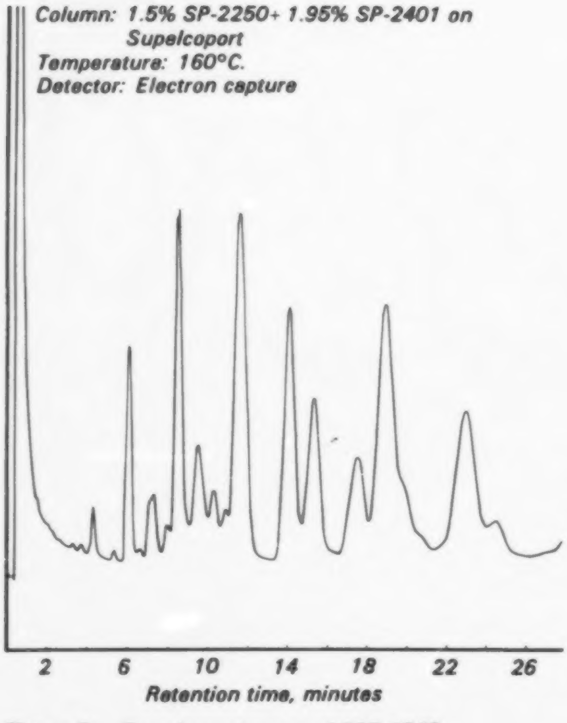


Figure 8. Gas chromatogram of PCB-1248.

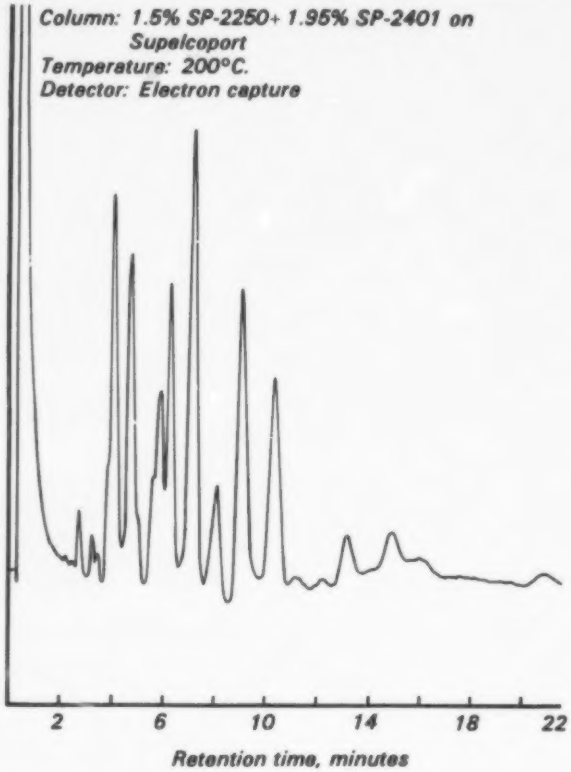


Figure 9. Gas chromatogram of PCB-1254.

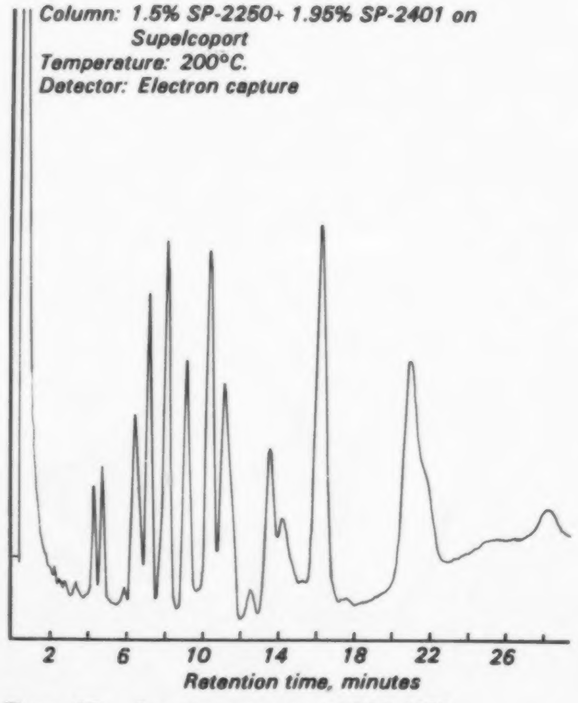


Figure 10. Gas chromatogram of PCB-1260.

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Nitroaromatics and Isophorone — Method 609

1. Scope and Application

1.1 This method covers the determination of certain nitroaromatics and isophorone. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
2,4-Dinitrotoluene	34611	121-14-2
2,6-Dinitrotoluene	34626	606-20-2
Isophorone	34408	78-59-1
Nitrobenzene	34447	98-95-3

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second gas chromatographic column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL defined in Section 14.1)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from those listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in method 606, 608, 611 and 612. Thus, a single sample may be extracted to measure all of the parameters included in the scope of each of these methods. When cleanup is required the concentration levels must be high enough to permit selection of aliquots of the extract, as necessary, to apply appropriate cleanup procedures. The analyst is allowed the latitude, under Gas Chromatography (Section 12), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.5 Any modifications of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is extracted with methylene chloride using separatory funnel techniques. The extract is dried and exchanged to hexane during concentration to 1.0 mL by evaporation. Isophorone and nitrobenzene are measured by flame ionization gas chromatography (FIDGC). The dinitrotoluenes are measured by electron capture GC (ECGC)(2).

2.2 The method provides a Florisil chromatographic cleanup procedure to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned(3). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400 °C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Thorough rinsing with such solvents usually eliminates PCB interferences. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

4. Safety

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(4-6) for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4 °C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel—2000-mL, with Teflon stopcock.

5.2.2 Drying column—Chromatographic column approximately 400 mm long × 19 mm ID with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish—Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish—Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials—Amber glass, 10- to 15-mL capacity, with Teflon-lined screw-cap.

5.2.8 Chromatographic column—100 mm long × 10 mm ID, Teflon stopcock.

5.3 Boiling chips—approximately 10/40 mesh. Heat to 400 °C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (± 2 °C). The bath should be used in a hood.

5.5 Balance—Analytical, capable of accurately weighing 0.0001 g.

5.6 Gas chromatograph—Analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1—1.2 m (4 ft.) long × 2 mm or 4 mm ID, Pyrex glass, packed with Gas-Chrom Q (80/100 mesh) coated with 1.95% QF-1/1.5% OV-17. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 12.1.

5.6.2 Column 2—3.0 m (10 ft.) long × 2 or 4 mm ID, Pyrex glass, packed with Gas-Chrom Q (80/100 mesh) coated with 3% OV-101.

5.6.3 Detector—Flame ionization. This detector has proven effective in the analysis of wastewaters for isophorone and nitrobenzene, and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1.

5.6.4 Detector—Electron capture. This detector has proven effective in the analysis of wastewaters for the dinitrotoluenes, and was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium hydroxide solution (10 N)—(ACS) Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sulfuric acid solution (1 + 1)—(ACS) Slowly, add 50 mL H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.4 Acetone, hexane, methanol, methylene chloride,—Pesticide quality or equivalent.

6.5 Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400 °C for 4 hours in a shallow tray.

6.6 Florisil—PR grade (60/100 mesh), purchase activated at 1250 °F and store in dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 200 °C in glass containers loosely covered with foil.

6.7 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality hexane, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4 °C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Systems Laboratory, Cincinnati, Ohio 45268.

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish gas chromatographic operating conditions to produce resolution of the parameters equivalent to those indicated in Table 1. The gas chromatographic system may be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane. One of the external standards should be at a concentration near, but above, the MDL limit and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

Eq. 1. $RF = (A_sC_{is})/(A_{is}C_s)$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard (µg/L).

C_s = Concentration of the parameter to be measured (µg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard.

Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X - R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before an samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3s
Lower Control Limit (LCL) = R − 3s

where R and s are calculated as in Section 8.2.3.

The UCL and LCL can be used to construct control charts(7) that are useful in observing trends in performance. The control limits above be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(7).

8.4. The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(8) should be followed, except that the bottle must not be prerinsed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4 °C from the time of collection until extraction.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction(2).

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Check the pH of the sample with wide-range pH paper and adjust to within the range of 5 to 9 with diluted sodium hydroxide or sulfuric acid.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Sections 10.7 and 10.8 describe a procedure for exchanging the methylene chloride solvent to hexane while concentrating the extract volume to 1.0 mL. When it is not necessary to achieve the MDL in Table 2, the solvent exchange may be made by the addition of 50 mL of hexane and concentration to 10 mL as described in method 606, Section 10.7.

10.7 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation.

10.8 Add 1 to 2 mL of hexane to the concentrator tube, and a clean boiling chip. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of hexane to the top. Place this micro K-D apparatus on a water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of hexane. Adjust the final volume to 1.0 mL and stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. Unless the sample is known to require cleanup, proceed with gas chromatographic analysis.

10.9 Determine the original sample volume by refilling the sample bottle to the mark with water and measuring the volume in a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Prepare a slurry of 10g of activated Florisil in methylene chloride in hexane (1 + 9)(V/V). Use it to pack a 10-mm ID chromatography column, gently tapping the column to settle the Florisil. Add 1 cm of anhydrous sodium sulfate to the top of the Florisil.

11.2.1 Just prior to exposure of the sodium sulfate layer to the air transfer the 1 mL of sample extract onto the column using an additional 2 mL of hexane to complete the transfer.

11.2.2 Just prior to exposure of the sodium sulfate layer to the air, add 30 mL of methylene chloride in hexane (1 + 9)(V/V) and continue the elution of the column. Elution of the column should be at a rate of about 2 mL skill per min. Discard the eluate from this fraction.

11.2.3 Next elute the column with 30 mL of acetone/methylene chloride (1 + 9)(V/V) into a 500-mL K-D flask equipped with a 10-mL concentrator tube. Concentrate the collected fraction by the K-D technique prescribed in Sections 10.6, 10.7, and 10.8 including the solvent exchange in 1 mL of hexane. This fraction should contain the nitroaromatics and isophorone.

11.2.4 Analyze by gas chromatography.

12. Gas Chromatography

12.1 Isophorone and nitrobenzene are analyzed by injection of a portion of the extract into an FIDGC. The dinitrotoluenes are analyzed by a separate injection into an ECGC. Table 1 summarizes some recommended gas chromatographic column materials and operating conditions for the instruments. This Table includes retention times and MDL obtained under these conditions. Examples of the parameter separations achieved by Column 1 are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, the analyst must not add the internal standard to sample the extract until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 2 to 5 μL of the sample extract using the solvent-flush technique[9]. Smaller (1.0 μL) volumes can be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 μL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected (μL).
V_t = Volume of total extract (μL).
V_s = Volume of water extracted (mL).

calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

Eq. 3

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract (μg).
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 Method detection limits—The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero[1]. The MDL concentrations listed in Table 1 were obtained using reagent water[10]. Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of recovery from reagent water and has been demonstrated to be applicable over the concentration range from 7 × MDL to 1000 × MDL[10].

14.3 In a single laboratory (Battelle, Columbus Laboratories), using spiked wastewater samples, the average recoveries presented in Table 2 were obtained[2]. Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the percent recovery is also included in Table 2.

14.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. "Determination of Nitroaromatics and Isophorone in Industrial and Municipal Wastewaters." Report for EPA Contract 68-03-2624 (In preparation).
3. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
4. "Carcinogens—Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
5. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
7. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
8. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
9. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).
10. "Determination of Method Detection Limit and Analytical Curve for EPA Methods 609—Nitroaromatics and Isophorone," special letter report for EPA Contract 68-03-2624. Environmental Monitoring and Support Laboratory—Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	Retention Time (min.)		Method Detection Limit (µg/L)	
	Column 1	Column 2	EC	FID
Nitrobenzene	3.31	4.31	13.7	3.6
2,6-Dinitrotoluene	3.52	4.75	0.01	—
Isophorone	4.49	5.72	15.7	5.7
2,4-Dinitrotoluene	5.35	6.54	0.02	—

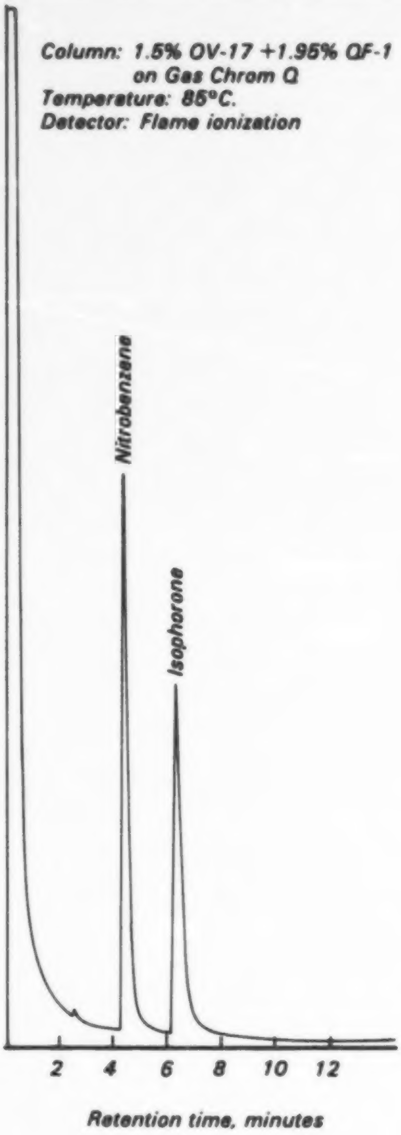
Column 1 conditions: Gas-Chrom Q (80/100 mesh) coated with 1.95% QF-1/1.5% OV-17 packed in a pyrex glass column 1.2 m (4 ft) long × 2 mm or 4 mm ID. Nitrogen carrier gas at a flow rate of 44 mL/min was used when determining isophorone and nitrobenzene by FID. The Column temperature was isothermal at 85°C. Methane (10%)/Argon (90%) carrier gas at flow rate of 44 mL/min was used when determining the dinitrotoluenes by ECGC. The column temperature was isothermal at 145°C.

Column 2 conditions: Gas-Chrom Q (80/100 mesh) coated with 3% OV-1 packed in a pyrex glass column 3.0 m (10 ft) long × 2 mm or 4 mm ID. Nitrogen carrier gas at a flow rate of 44 mL/min was used when determining isophorone and nitrobenzene by FID. The column temperature was isothermal at 100°C. Methane (10%)/Argon (90%) carrier gas flow rate of 44 mL/min was used when determining the dinitroltoluenes by ECGC. The column temperature was isothermal, 150°C.

A 2 mm ID column was used with the FIDGC and a 4 mm ID column was used with the ECGC.

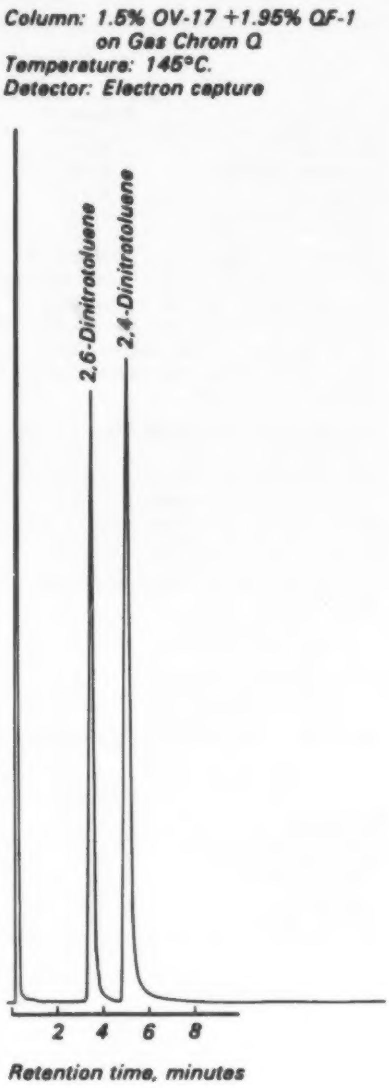
Table 2. *Single Operator Accuracy and Precision*

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (µg/L)	Number of Analyses	Matrix Types
2,4-Dinitrotoluene	63	3.1	5-100	21	4
2,6-Dinitrotoluene	66	3.2	5-50	24	4
Isophorone	73	4.6	50-60	21	4
Nitrobenzene	71	5.9	90-100	24	4



Column: 1.5% OV-17 +1.95% QF-1
on Gas Chrom Q
Temperature: 85°C.
Detector: Flame ionization

Figure 1. Gas chromatogram of nitrobenzene and isophorone.



Column: 1.5% OV-17 +1.95% QF-1
on Gas Chrom Q
Temperature: 145°C.
Detector: Electron capture

Figure 2. Gas chromatogram of dinitrotoluenes.

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Polynuclear Aromatic Hydrocarbons — Method 610

1. Scope and Application

1.1 This method covers the determination of certain polynuclear aromatic hydrocarbons (PAH). The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Acenaphthene	34205	83-32-9
Acenaphthylene	34200	208-96-8
Anthracene	34220	120-12-7
Benzo (a) anthracene	34526	56-55-3
Benzo (a) pyrene	34247	50-32-8
Benzo (b) fluoranthene	34230	205-99-2
Benzo (ghi) perylene	34521	191-24-2
Benzo (k) fluoranthene	34242	207-08-9
Chrysene	34320	218-01-9
Dibenzo (a, h) anthracene	34556	53-70-3
Fluoranthene	34376	206-44-0
Fluorene	34381	86-73-7
Indeno (1, 2, 3-cd) pyrene	34403	193-39-5
Naphthalene	34696	91-20-3
Phenanthrene	34461	85-01-8
Pyrene	34469	129-00-0

1.2 This is a chromatographic method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for many of the parameters listed in Section 1.1, using the extract produced by this method.

1.3 This method provides for both high performance liquid chromatographic (HPLC) and gas chromatographic (GC) approaches to the determination of PAHs. The gas chromatographic procedure does not adequately resolve the following four pairs of compounds: anthracene and phenanthrene; chrysene and benzo (a) anthracene; benzo (b) fluoranthene and benzo (k) fluoranthene; and dibenzo (a, h) anthracene and indeno (1, 2, 3-cd) pyrene. Unless the purpose for the analysis can be served by reporting the sum of an unresolved pair, the liquid chromatographic approach must be used for these compounds. The liquid chromatographic method does resolve all 16 of the PAHs listed.

1.4 The method detection limit (MDL, defined in Section 15)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ depending upon the nature of interferences in the sample matrix.

1.5 The sample extraction and concentration steps in this method are essentially the same as in methods 606, 608, 609, 611 and 612. Therefore, a single sample may be extracted to measure the parameters included in the scope of each of these methods, provided the concentration is high enough to permit selecting aliquots of the extract for cleanup, when required. Selection of the aliquots must be made prior to the solvent exchange steps of this method. The analyst is allowed the latitude, under Gas Chromatography (Section 13), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.6 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.7 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC and GC and in the interpretation of liquid and gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and concentrated to a volume of 10 mL or less. The solvent is exchanged to cyclohexane prior to cleanup. Following cleanup, when using HPLC for determination of the PAHs, the solvent is exchanged to acetonitrile. Ultraviolet (UV) and fluorescence detectors are used with HPLC. When cleanup is not required and when flame ionization detector GC is used for determination, the methylene chloride extract may be analyzed directly. When cleanup is required, the cyclohexane exchange is made. Instrumental conditions are described which permit the separation and measurement of the PAH compounds(2).

2.2 A silica gel column cleanup procedure is provided to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned(3). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

3.3 The extent of interferences that may be encountered using liquid chromatographic techniques has not been fully assessed. Although the HPLC conditions described allow for a unique resolution of the specific PAH compounds covered by this method, other PAH compounds may interfere.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(4-6) for the information of the analyst.

4.2 The following paramenters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens; benzo (a) anthracene, benzo (a) pyrene and dibenzo (a, h) anthracene.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials - Amber glass, 10- to 15- mL capacity, with Teflon-lined screwcap.

5.2.8 Chromatographic column - 250 mm long x 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.3 Boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

5.5 Balance — Analytical, capable of accurately weighing 0.0001g.

5.6 High performance liquid chromatographic apparatus (modular):

5.6.1 Gradient pumping system, constant flow.

5.6.2 Reverse phase column, 5 micron HC-ODS Sil-X, 250 mm x 2.6 mm ID (Perkin-Elmer No. 089-0716 or equivalent).

5.6.3 Fluorescence detector, for excitation at 280 nm and emission greater than 389 nm cutoff (Corning 3-75 or equivalent). Fluorometers should have dispersive optics for excitation and can utilize either filter or dispersive optics at the emission detector.

5.6.4 UV detector, 254 nm, coupled to fluorescence detector.

5.6.5 Strip-chart recorder compatible with detectors. Use of a data system for measuring peak areas and retention times is recommended.

5.7 Gas chromatograph - An analytical system complete with temperature programmable gas chromatograph suitable for on-column injection or splitless injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.7.1 Column - 1.8 m long x 2 mm ID pyrex glass packed with 3% OV-17 on Chromosorb W-AW-DCMS (100/120 mesh) or equivalent. This column was used to develop the retention time data in Table 2. Guidelines for the use of alternate column packings are provided in Section 13.

5.7.2 Detector - Flame ionization. This detector has proven effective in the analysis of wastewaters for the compounds listed in the scope excluding the four pairs of unresolved compounds listed in Section 1.3. Guidelines for the use of alternate detectors are provided in Section 12.2.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium thiosulfate - (ACS) Granular.

6.3 Cyclohexane, methanol, acetone, methylene chloride, and pentane - Pesticide quality or equivalent.

6.4 Acetonitrile, high purity HPLC quality, distilled in glass.

6.5 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.

6.6 Silica gel - Grade 923 (100/200 mesh) dessicant (Davison Chemical or equivalent). Before use, activate for at least 16 hours at 130°C in a shallow glass tray, loosely covered with foil.

6.7 Stock standard solutions (1.00 μg/μL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material, Dissolve the material in HPLC quality acetonitrile, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish liquid or gas chromatographic operating parameters to produce resolution of the parameters equivalent to that indicated in Tables 1 or 2. The chromatographic system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetonitrile. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Analyze each calibration standard (5 to 25 μL for HPLC and 2 to 5 μL for GC), and tabulate peak height or area responses against the mass injected. The results may be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with acetonitrile. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Analyze each calibration standard (5 to 25 µL for HPLC and 2 to 5 µL for GC) and tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using Equation 1.

$$\text{Eq. 1} \quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard, (µg/L).
C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant (< 10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetronitrile 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 3, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X\text{-}R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R — 3 s

where R and s are calculated as in Section 8.2.3.

The UCL and LCL can be used to construct control charts[7] that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly[7].

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 14.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as chromatography with a dissimilar column or detector must be used. This may include the use of a mass spectrometer. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices[8] should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon tubing and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. PAHs are known to be light sensitive, therefore, samples, extracts and standards should be stored in amber or foil wrapped bottles in order to minimize photolytic decomposition. Fill the sample bottle and, if residual chlorine is present, add 80 mg of sodium thiosulfate per liter of sample. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used for measurement of residual chlorine[9]. Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days, and analysis completed within 40 days of extraction[2].

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of ten minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle, rinse and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommemded for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles and protected from light.

10.7 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Before the silica gel cleanup technique can be utilized, the extract solvent must be exchanged to cyclohexane. Add a 1- to 10- mL aliquot of sample extract (in methylene chloride) and a boiling chip to a clean K-D concentrator tube. Add 4 mL cyclohexane and attach a micro-Snyder column. Prewet the micro-Snyder column by adding 0.5 mL methylene chloride to the top. Place the micro-K-D apparatus on a boiling (100°C) water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of the liquid reaches 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a minimum of cyclohexane. Adjust the extract volume to about 2 mL.

11.3 Silica gel column cleanup for PAHs.

11.3.1 Prepare a slurry of 10g activated silica gel in methylene chloride and place this in a 10-mm ID chromatography column. Gently tap the column to settle the silica gel and elute the methylene chloride. Add 1 to 2 cm of anhydrous sodium sulfate to the top of the silica gel.

11.3.2 Preelute the column with 40 mL of pentane. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the 2 mL of cyclohexane sample extract onto the column, using an additional 2 mL of cyclohexane to complete the transfer.

11.3.3 Just prior to exposure of the sodium sulfate layer to the air, add 25 mL pentane and continue elution of the column. Discard the pentane eluate.

11.3.4 Elute the column with 25 mL of methylene chloride/pentane (4 + 6) (V/V) and collect the eluate in a 500-mL K-D flask equipped with a 10-mL concentrator tube. Elution of the column should be at a rate of about 2 mL/min.

11.3.5 Concentrate the collected fraction to less than 10 mL by K-D techniques as in Section 10.6, using pentane to rinse the walls of the glassware. Proceed with HPLC or GC analysis.

12. High Performance Liquid Chromatography (HPLC)

12.1 To the extract in the concentrator tube, add 4 mL of acetonitrile and a new boiling chip, then attach a micro-Snyder column. Increase the temperature of the hot water bath to 95 to 100°C. Concentrate the solvent as in Section 10. After cooling, remove the micro-Snyder column and rinse its lower joint into the concentrator tube with about 0.2 mL acetonitrile. Adjust the extract volume to 1.0 mL.

12.2 Table 1 summarizes the recommended HPLC column materials and operating conditions for the instrument. This table includes retention times, capacity factors, and MDL that were obtained under these conditions. The UV detector is recommended for the determination of naphthalene, acenaphthylene, acenapthene, and fluorene, and the fluorescence detector is recommended for the remaining PAHs. Examples of the parameter separations achieved by this HPLC column are shown in Figures 1 and 2. Other HPLC columns, chromatrograpic conditions or detectors may be used if the requirements of Section 8.2 are met.

12.3 Calibrate the system daily as described in Section 7.

12.4 If the internal standard approach is being used, the internal standard must be added to sample extract and mixed thoroughly, immediately, before injection into the instrument.

12.5 Inject 5 to 25 μL of the sample extract using a high pressure syringe or a constant volume sample injection loop. Record the volume injected to the nearest 0.1 μL, and the resulting peak size in height or area units. Re-equilibrate the liquid chromatographic column at the initial gradient conditions for at least 10 minutes between injections.

12.6 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.7 If the peak height or area exceeds the linear range of the system, dilute the extract with acetonitrile and reanalyze.

12.8 If the peak area measurement is prevented by the presence of interferences, further cleanup is required.

13. Gas Chromatography

13.1 The packed column GC procedure will not resolve certain isomeric pairs as indicated in Section 1.3 and Table 2. The liquid chromatographic procedure (Section 12) must be used for these materials. Capillary (open-tubular) columns may be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

13.2 To achieve maximum sensitivity with this method, the extract must be concentrated to 1.0 mL. Add a clean boiling chip to the methylene chloride extract in the concentrator tube. Attach a two-ball micro-Snyder column. Prewet the micro-Snyder column by adding about 0.5 mL of methylene chloride to the top. Place the micro K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches 0.5 mL, remove the K-D apparatus. Drain and cool for at least 10 minutes. Remove the micro-Snyder column and rinse its lower joint into the concentrator tube with a small volume of methylene chloride. Adjust the final volume to 1.0 mL and stopper the concentrator tube.

13.3 Table 2 describes the recommended GC column and operating conditions for the instrument. This table includes retention times that were obtained under these conditions. An example of the parameter separations achieved by this column is shown in Figure 3. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

13.4 Calibrate the GC system daily as described in Section 7.

13.5 If the internal standard approach is being used, add the internal standard to sample extract and mix thoroughly, immediately, before injection into the instrument.

13.6 Inject 2 to 5 µL of the sample extract using the solvent-flush technique(10). Smaller (1.0 µL) volumes may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

13.7 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

13.8 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

13.9 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

14. Calculations

14.1 Determine the concentration of individual parameters in the sample.

14.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from Equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected (µL).
V_t = Volume of total extract (µL).
V_s = Volume of water extracted (mL).

14.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and Equation 3.

$$\text{Eq. 3. Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract (µg).
V_o = Volume of water extracted, in liters.

14.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

14.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits established in Section 8.4, data for the affected parameters must be labeled as suspect.

15. Method Performance

15.1 Method detection limits - The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(11). Similar results were achieved using representative wastewaters. MDL for the GC approach were not determined.

15.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable over the concentration range from 8 x MDL to 800 x MDL(11), with the following exception: benzo(ghi)perylene recovery at 80 x and 800 x MDL were low (35% and 45% respectively).

15.3 In a single laboratory (Battelle Columbus Laboratories), using spiked wastewater samples, the average recoveries presented in Table 3 were obtained(2). Each spiked sample was analyzed in triplicate on two separate days. The standard deviation of the precent recovery is also included in Table 3.

15.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. Determination of Polynuclear Aromatic Hydrocarbons in Industrial and Municipal Wastewaters," Report for EPA Contract 68-03-2624 (In preparation).
3. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
4. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
5. "OSHA Safety and Health Standards, General Industry," (29CFR-1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
7. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
8. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

9. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600-4/79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

10. Burke, J. A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,*1037 (1965).

11. Cole, T., Riggins, R., and Glaser, J., "Evaluation of Method Detection Limits and Analytical Curve for EPA Method 610 - PNAs," International Symposium on Polynuclear Aromatic Hydrocarbons, 5th, Battelle Columbus Laboratory, Columbus, Ohio (1980).

Table 1. *High Performance Liquid Chromatography Conditions and Method Detection Limits*

Parameter	Retention Time (min)	Capacity Factor (k')	Method Detection Limit (µg/L)[a]
Naphthalene	16.6	12.2	1.8
Acenaphthylene	18.5	13.7	2.3
Acenaphthene	20.5	15.2	1.8
Fluorene	21.2	15.8	0.21
Phenanthrene	22.1	16.6	0.64
Anthracene	23.4	17.6	0.66
Fluoranthene	24.5	18.5	0.21
Pyrene	25.4	19.1	0.27
Benzo(a)anthracene	28.5	21.6	0.013
Chrysene	29.3	22.2	0.15
Benzo(b)fluoranthene	31.6	24.0	0.018
Benzo(k)flouranthene	32.9	25.1	0.017
Benzo(a)pyrene	33.9	25.9	0.023
Dibenzo(a,h)anthracene	35.7	27.4	0.030
Benzo(ghi)perylene	36.3	27.8	0.076
Indeno(1,2,3-cd)pyrene	37.4	28.7	0.043

HPLC conditions: Reverse phase HC-ODS Sil-X 2.6 mm x 250 mm Perkin-Elmer column; isocratic elution for 5 min using acetonitrile/water (4 + 6), then linear gradient elution to 100% acetonitrile over 25 minutes; flow rate is 0.5 mL/min. If columns having other internal diameters are used, the flow rate should be adjusted to maintain a linear velocity of 2 mm/sec.

[a] The method detection limit for naphthalene, acenaphthylene, acenaphthene, and fluorene were determined using a UV detector. All others were determined using a fluorescence detector.

Table 2. *Gas Chromatographic Operating Conditions and Retention Times*

Parameter	Retention Time (min)
Naphthalene	4.5
Acenaphthylene	10.4
Acenaphthene	10.8
Fluorene	12.6
Phenanthrene	15.9
Anthracene	15.9
Fluoranthene	19.8
Pyrene	20.6
Benzo(a)anthracene	20.6
Chrysene	24.7
Benzo(b)fluoranthene	28.0
Benzo(k)fluoranthene	28.0
Benzo(a)pyrene	29.4
Dibenzo(a,h)anthracene	36.2
Indeno(1,2,3-cd)pyrene	36.2
Benzo(ghi)perylene	38.6

GC conditions: Chromosorb W-AW-DCMS (100/120 mesh) coated with 3% OV-17, packed in a 1.8 m long x 2 mm ID glass column, with nitrogen carrier gas at a flow rate of 40 mL/min. Column temperature was held at 100°C for 4 min, then programmed at 8°/minute to a final hold at 280°C.

Table 3　*Single Operator Accuracy and Precision*

Parameter	*Average Percent Recovery*	*Standard Deviation %*	*Spike Range (µg/L)*	*Number of Analyses*	*Matrix Types*
Acenaphthene	*88*	*5.7*	*11.6-25*	*24*	*4*
Acenaphthylene	*93*	*6.4*	*250-450*	*24*	*4*
Anthracene	*93*	*6.3*	*7.9-11.3*	*24*	*4*
Benzo(a)anthracene	*89*	*6.9*	*0.64-0.66*	*24*	*4*
Benzo(a)pyrene	*94*	*7.4*	*0.21-0.30*	*24*	*4*
Benzo(b)fluoranthene	*97*	*12.9*	*0.24-0.30*	*24*	*4*
Benzo(ghi)perylene	*86*	*7.3*	*0.42-3.4*	*24*	*4*
Benzo(k)fluoranthene	*94*	*9.5*	*0.14-6.2*	*24*	*4*
Chrysene	*88*	*9.0*	*2.0-6.8*	*24*	*4*
Dibenzo(a,h)anthracene	*87*	*5.8*	*0.4-1.7*	*24*	*4*
Fluoranthene	*116*	*9.7*	*0.3-2.2*	*24*	*4*
Fluorene	*90*	*7.9*	*6.1-23*	*24*	*4*
Indeno(1,2,3-cd)pyrene	*94*	*6.4*	*0.96-1.4*	*24*	*4*
Naphthalene	*78*	*8.3*	*20-70*	*24*	*4*
Phenanthrene	*98*	*8.4*	*3.8-5.0*	*24*	*4*
Pyrene	*96*	*8.5*	*2.3-6.9*	*24*	*4*

***Column:* HC-ODS SIL-X**
***Mobile phase:* 40% to 100% Acetonitrile in water**
***Detector:* Ultra violet at 254nm**

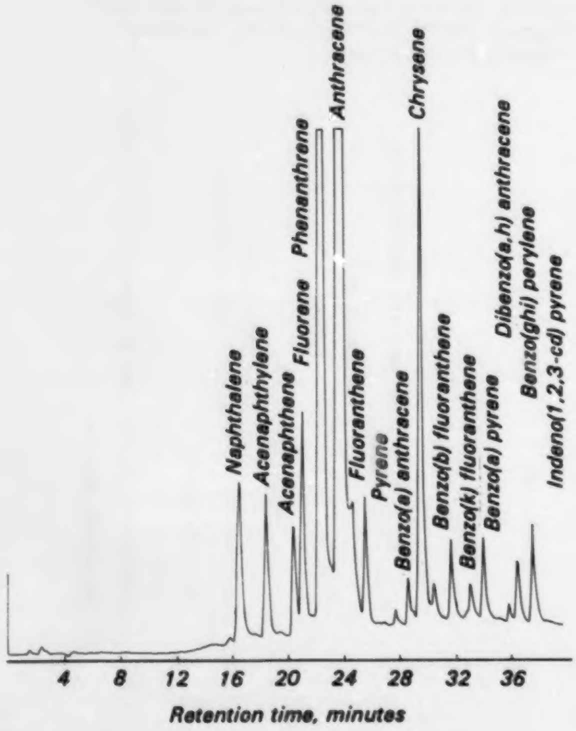


Figure 1. ***Liquid chromatogram of polynuclear aromatic hydrocarbons.***

Column: HC-ODS SIL-X
Mobile phase: 40% to 100% Acetonitrile in water
Detector: Fluorescence

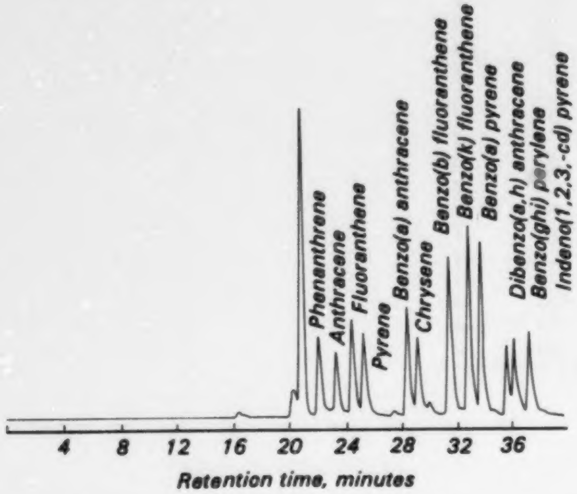


Figure 2. *Liquid chromatogram of polynuclear aromatic hydrocarbons.*

Column: 3% OV-17 on Chromosorb W-AW-DCMS
Program: 100°C. 4 min.,8° per min. to 280°C.
Detector: Flame ionization

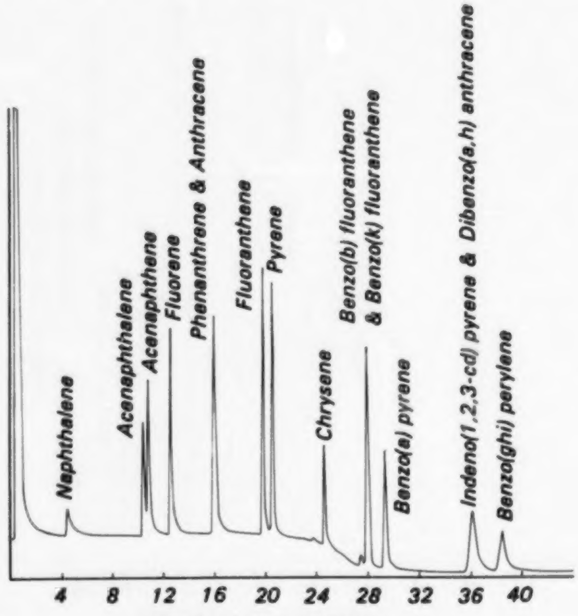


Figure 3. *Gas chromatogram of polynuclear aromatic hydrocarbons.*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Haloethers — Method 611

1. Scope and Application

1.1 This method covers the determination of certain haloethers. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
Bis(2-chloroethyl) ether	34273	111-44-4
Bis(2-chloroethoxy) methane	34278	111-91-1
Bis(2-chloroisopropyl) ether	34283	108-60-1
4-Bromophenyl phenyl ether	34636	101-55-3
4-Chlorophenyl phenyl ether	34641	7005-72-3

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. This method describes analytical conditions for a second GC column that can be used to confirm measurements made with the primary column. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract from this method.

1.3 The method detection limit (MDL, defined in Section 14.1)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from that listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in methods 606, 608, 609, and 612. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, to apply appropriate cleanup procedures. The analyst is allowed the latitude, under Gas Chromatography (Section 12), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and exchanged to hexane during concentration to a volume of 10 mL or less. GC conditions are described which permit the separation and measurement of the compounds in the extract using a halide specific detector[2].

2.2 The method provides a Florisil column cleanup procedure to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.[3] Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and reagent water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the MDL listed in Table 1.

3.3 Dichlorobenzenes are known to coelute with haloethers under some gas chromatographic conditions. If these materials are present together in a sample, it may be necessary to analyze the extract with two different column packings to completely resolve all of the compounds.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified[4-8] for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID, with coarse frit.

5.2.3 Chromatographic column - 400-mm long x 19 mm ID glass with coarse fritted plate on bottom and Teflon stopcock (Kontes K-420540-0224 or equivalent).

5.2.4 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.5 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.6 Snyder column, Kuderna-Danish - Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.7 Vials - Amber glass, 10- to 15- mL capacity, with Teflon-lined screwcap.

5.3 Boiling chips - Approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (±2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.6 Gas chromatograph - An analytical system complete with temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, detector, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column 1 - 1.8 m long x 2 mm ID pyrex glass, packed with Supelcoport, (100/120 mesh) coated with 3% SP-1000 or equivalent. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 12.1.

5.6.2 Column 2 - 1.8 m long x 2 mm ID pyrex glass, packed with Tenax-GC (60/80 mesh) or equivalent.

5.6.3 Detector - Halide specific: electrolytic conductivity or microcoulometric. These detectors have proven effective in the analysis of wastewaters for the parameters listed in the scope of this method. The Hall conductivity detector was used to develop the method performance statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1. Although less selective, an electron capture detector is an acceptable alternative.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Sodium thiosulfate - (ACS) Granular.

6.3 Acetone, methanol, methylene chloride, hexane, and petroleum ether (boiling range 30 to 60°C) - Pesticide quality or equivalent.

6.4 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.

6.5 Florisil - PR Grade (60/100 mesh); purchase activated at 1250°F and store in the dark in glass container with glass stoppers or foil-lined screw caps. Before use, activate each batch overnight at 130°C in a foil-covered glass container.

6.6 Ethyl ether - Nanograde, redistilled in glass, if necessary.

6.6.1 Must be free of peroxides as indicated by EM Laboratories Quant test strips. (Available from Scientific Products Co., Cat. No. P1126-8, and other suppliers.)

6.6.2 Procedures recommended for removal of peroxides are provided with the test strips. After cleanup 20 mL ethyl alcohol preservative must be added to each liter of ether.

6.7 Stock standard solutions (1.00 µg/µL) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality acetone and dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available for the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.7.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish gas chromatographic operating parameters to produce retention times equivalent to those listed in Table 1. The GC chromatographic system may be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with hexane. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with hexane. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 µL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1}\quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
C_{is} = Concentration of the internal standard, (µg/L).
C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant ($<$ 10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

7.5 The cleanup procedure in Section 11 utilizes Florisil column chromatography. Florisil from different batches or sources may vary in adsorption capacity. To standardize the amount of Florisil which is used, the use of lauric acid value(7) is suggested. The referenced procedure determines the adsorption from hexane solution of lauric acid (mg) per gram Florisil. The amount of Florisil to be used for each column is calculated by dividing 110 by this ratio and multiplying by 20 g.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X\text{-}R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = R + 3 s
Lower Control Limit (LCL) = R — 3 s

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(8) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(8)

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2 If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confirmatory techniques such as GC with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(9) should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be a free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction. Fill the sample bottles and, if residual chlorine is present, add 80 mg of sodium thiosulfate per each liter of water. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used to measure the residual chlorine(10). Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction(2).

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner walls. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60° to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus and allow it to drain and cool for at least 10 minutes.

NOTE: Some of the haloethers are very volatile and significant losses will occur in concentration steps if care is not exercised. It is important to maintain a constant gentle evaporation rate and not to allow the liquid volume to fall below 1 to 2 mL before removing the K-D from the hot water bath.

10.7 Momentarily remove the Snyder column, add 50 mL of hexane and a new boiling chip and replace the column. Raise the temperature of the water bath to 85 to 90°C. Concentrate the extract as in Section 10.6 except use hexane to prewet the column. When the apparent volume of liquid reaches 1 to 2 mL, remove the K-D and allow it to drain and cool at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles.

10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedure recommended in this method has been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Florisil column cleanup for haloethers:

11.2.1 Adjust the sample extract volume to 10 mL.

11.2.2 Place a charge (nominally 20 g, actual amount determined as in Section 7.5) of activated Florisil in a 19-mm ID chromatographic column. After settling the Florisil by tapping the column, add about one-half inch layer of anhydrous granular sodium sulfate to the top. Allow the Florisil to cool.

11.2.3 Pre-elute the column with 50 to 60 mL of petroleum ether. Discard the eluate and just prior to exposure of the sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Discard the eluate. Just prior to exposure of the sodium sulfate layer to the air, begin eluting the column with 300 mL of ethyl ether/petroleum ether (6 + 94) (V/V). Adjust the elution rate to approximately 5 mL/min and collect the eluate in a 500-mL K-D flask equipped with a 10-mL concentrator tube. This fraction should contain all of the haloethers.

11.2.4 Concentrate the fraction by K-D as in Section 10.6 except prewet the Snyder column with hexane. When the apparatus is cool, remove the column and rinse the flask and its lower joint into the concentrator tube with hexane. Adjust the volume to 10 mL. Analyze by GC (Section 12.)

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. This table includes retention times and MDL that were obtained under these conditions. Examples of the parameter separations achieved by these columns are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, the analyst must not add the internal standard to sample extracts until immediately before injection into the instrument. Mix thoroughly.

12.4 Inject 2 to 5 µL of the sample extract using the solvent-flush technique[11]. Smaller (1.0 µL) volumes can be injected if automatic devices are employed. Record the extract volume to the nearest 0.1 mL and the volume injected to the nearest 0.05 µL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2. Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected (µL).
V_t = Volume of total extract (µL).
V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

$$\text{Eq. 3. Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract (µg).
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.4, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero[1]. The MDL concentrations listed in Table 1 were obtained using reagent water[12]. Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of recovery from spiked reagent water and has been demonstrated to be applicable for the concentration range from 4X MDL to 1000 x MDL[12].

14.3 In a single laboratory (Monsanto Research Center), using spiked wastewater samples, the average recoveries presented in Table 2 were obtained[2]. Each spiked sample was analyzed in triplicate on three separate occasions. The standard deviation of the percent recovery is also included in Table 2.

14.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. "Determination of Haloethers in Industrial and Municipal Wastewaters." Report for EPA Contract 68-03-2633 (In preparation).
3. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
4. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
5. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

7. Mills, P.A., "Variation of Florisil Activity: Simple Method for Measuring Absorbent Capacity and Its Use in Standardizing Florisil Columns," *Journal of the Association of Official Analytical Chemists, 51,* 29 (1968).

8. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

9. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

10. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600-4/79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

11. Burke, J. A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).

12. "EPA Method Validation Study 21 Method 611 (Haloethers)," Report for EPA Contract 68-03-2633 (In Preparation).

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	*Retention Time (min.)*		*Method Detection Limit (µg/L)*
	Column 1	*Column 2*	
Bis(2-chloroisopropyl) ether	*8.4*	*9.7*	*0.8*
Bis(2-chloroethyl) ether	*9.3*	*9.1*	*0.3*
Bis(2-chloroethoxy) methane	*13.1*	*10.0*	*0.5*
4-Chlorophenyl phenyl ether	*19.4*	*15.0*	*3.9*
4-Bromophenyl phenyl ether	*21.2*	*16.2*	*2.3*

Column 1 conditions: Supelcoport (100/120 mesh) coated with 3% SP-1000 packed in 1.8 m long x 2 mm ID glass column with helium carrier gas at a flow rate of 40 mL/min. Column temperature: 60°C for 2 min after injection then program at 8°C/min to 230°C and hold for 4 min. Under these conditions the retention time for Aldrin is 22.6 min.

Column 2 conditions: Tenax-GC (60/80 mesh) packed in a 1.8 m long x 2mm ID glass column with helium carrier gas at 40 mL/min flow rate. Column temperature: 150°C for 4 min after injection then program at 16°C/min to 310°C. Under these conditions the retention time for Aldrin is 18.4 min.

Table 2. *Single Operator Accuracy and Precision*

Parameter	*Average Percent Recovery*	*Standard Deviation %*	*Spike Range (µg/L)*	*Number of Analyses*	*Matrix Types*
Bis(2-chloroethyl)ether	*59*	*4.5*	*97*	*27*	*3*
Bis(2-chloroethoxy)methane	*62*	*5.3*	*138*	*27*	*3*
Bis(2-chloroisopropyl)ether	*67*	*4.0*	*54*	*27*	*3*
4-Bromophenyl phenyl ether	*78*	*3.5*	*14*	*27*	*3*
4-Chlorophenyl phenyl ether	*73*	*4.5*	*30*	*27*	*3*

Column: 3% SP-1000 on Supelcoport
Program: 60°C.-2 minutes 8°/minute to 230°C.
Detector: Hall electrolytic conductivity

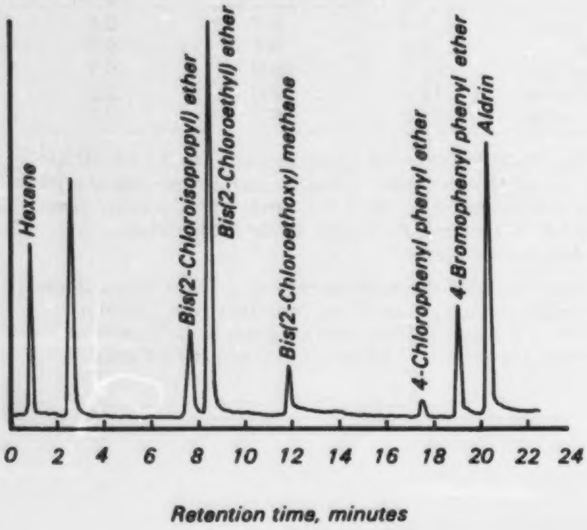


Figure 1. *Gas chromatogram of haloethers.*

Column: Tenax GC
Program: 150°C.-4 minutes 16°/minute to 310°C.
Detector: Hall electrolytic conductivity

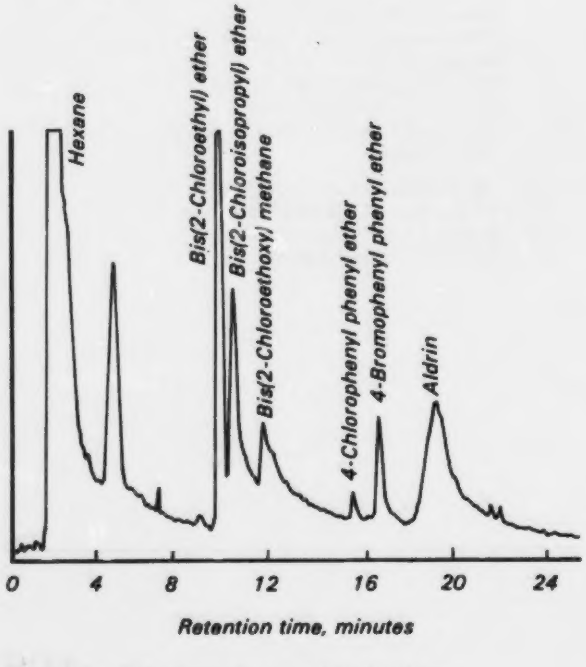


Figure 2. *Gas chromatogram of haloethers.*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Chlorinated Hydrocarbons — Method 612

1. Scope and Application

1.1 This method covers the determination of certain chlorinated hydrocarbons. The following parameters can be determined by this method:

Parameter	STORET No.	CAS No.
2-Chloronaphthalene	34581	91-58-7
1,2-Dichlorobenzene	34536	95-50-1
1,3-Dichlorobenzene	34566	541-73-1
1,4-Dichlorobenzene	34571	106-46-7
Hexachlorobenzene	39700	118-74-1
Hexachlorobutadiene	34391	87-68-3
Hexachlorocyclopentadiene	34386	77-47-4
Hexachloroethane	34396	67-72-1
1,2,4-Trichlorobenzene	34551	120-82-1

1.2 This is a gas chromatographic (GC) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1. When this method is used to analyze unfamiliar samples for any or all of the compounds above, compound identifications should be supported by at least one additional qualitative technique. Method 625 provides gas chromatograph/mass spectrometer (GC/MS) conditions appropriate for the qualitative and quantitative confirmation of results for all of the parameters listed above, using the extract produced by this method.

1.3 The method detection limit (MDL defined in Section 14.1)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater may differ from that listed, depending upon the nature of interferences in the sample matrix.

1.4 The sample extraction and concentration steps in this method are essentially the same as in methods 606, 608, 609, and 611. Thus, a single sample may be extracted to measure the parameters included in the scope of each of these methods. When cleanup is required, the concentration levels must be high enough to permit selecting aliquots, as necessary, to apply appropriate cleanup procedures. The analyst is allowed the latitude, under Gas Chromatography (Section 12), to select chromatographic conditions appropriate for the simultaneous measurement of combinations of these parameters, provided that the requirements of Section 8.2 are met.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of gas chromatography and in the interpretation of gas chromatograms. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is solvent extracted with methylene chloride using a separatory funnel. The methylene chloride extract is dried and solvent exchanged to hexane during concentration to a volume of 10 mL or less. GC conditions are described which permit the separation and measurement of the compounds in the extract using an ECD(2).

2.2 The method provides a Florisil column cleanup procedure to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in gas chromatograms. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned(3). Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. It should then be drained dry, and heated in a muffle furnace at 400°C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. The cleanup procedure in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches to achieve the detection limits listed in Table 1.

4. Safety

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(4-6) for the information of the analyst.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle - Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional) - Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4°C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel - 2000-mL, with Teflon stopcock.

5.2.2 Drying column - Chromatographic column 400 mm long x 19 mm ID with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish - 10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish - 500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish - three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish - two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials - Amber glass, 10- to 15- mL capacity, with Teflon- lined screwcap.

5.2.8 Chromatography column - 300 mm long x 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.3 Boiling chips - approximately 10/40 mesh. Heat to 400°C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath - Heated, with concentric ring cover, capable of temperature control (± 2°C). The bath should be used in a hood.

5.5 Balance - Analytical, capable of accurately weighing 0.0001 g.

5.6 Gas chromatograph - An analytical system complete with gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, gases, ECD, and strip-chart recorder. A data system is recommended for measuring peak areas.

5.6.1 Column - 1.8 m long x 2 mm ID pyrex glass, packed with 1.5% OV-1 + 2.4% OV-225 on Supelcoport (80/100 mesh) or equivalent. This column was used to develop the method performance statements in Section

14. Guidelines for the use of alternate column packings are provided in Section 12.1

5.6.2 Detector - Electron capture. This detector has proven effective in the analysis of wastewaters for the parameters listed in the scope, and was used to develop the accuracy and precision statements in Section 14. Guidelines for the use of alternate detectors are provided in Section 12.1.

6. Reagents

6.1 Reagent water - Reagent water is defined as a water in which an interferent is not observed at the MDL of each parameter of interest.

6.2 Acetone, methanol, methylene chloride, hexane, petroleum ether (Boiling range 30 to 60°C) - Pesticide quality or equivalent.

6.3 Sodium sulfate - (ACS) Granular, anhydrous. Purify by heating at 400°C for four hours in a shallow tray.

6.4 Florisil - PR grade (60/100 mesh); purchase activated at 1250°F and store in the dark in glass containers with glass stoppers or foil-lined screw caps. Before use, activate each batch at 130°C in foil-covered glass containers.

6.5 Stock standard solutions (1.00 $\mu g/\mu L$) - Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.5.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality isooctane, dilute to volume in a 10-mL volumetric flask. Larger volumes can be used at the convenience of the analyst. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.5.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store at 4°C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 45268.

6.5.3 Stock standard solutions must be replaced after six months, or sooner if comparison with check standards indicate a problem.

7. Calibration

7.1 Establish GC operating conditions to produce resolution of the parameters equivalent to those indicated in Table 1. The GC system may be calibrated using external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with isooctane. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.2.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against the mass injected. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (< 10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask. To each calibration standard, add a known constant amount of one or more internal standards, and dilute to volume with isooctane. One of the standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

7.3.2 Using injections of 2 to 5 μL of each calibration standard, tabulate peak height or area responses against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1 } RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = Response for the parameter to be measured.

A_{is} = Response for the internal standard.

C_{is} = Concentration of the internal standard, ($\mu g/L$).

C_s = Concentration of the parameter to be measured, ($\mu g/L$).

If the RF value over the working range is a constant (< 10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

7.4 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each compound to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X-R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = $R + 3s$
Lower Control Limit (LCL) = $R - 3s$

where R and s are calculated as in Section 8.2.3.

The UCL and LCL can be used to construct control charts(7) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as $R \pm s$. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(7).

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for a particular parameter does not fall within the control limits for method performance, the results reported for that parameter in all samples processed as part of the same set must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of one-liter aliquot of reagent water, that all glassware and reagents interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak on the chromatogram, confimatory techniques such as gas chromatography with a dissimilar column, specific element detector, or mass spectrometer must be used. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(8) should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4°C from the time of collection until extraction.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction[2].

10. Sample Extraction

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.3 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.4 Assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the Kuderna-Danish if the requirements of Section 8.2 are met.

10.5 Pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.6 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65°C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 to 2 mL, remove the K-D apparatus from the water bath and allow it to drain for at least 10 minutes while cooling.

NOTE: The dichlorobenzenes have a sufficiently high volatility that significant losses may occur in concentration steps if care is not exercised. It is important to maintain a constant gentle evaporation rate and not to allow the liquid volume to fall below 1 to 2 mL before removing the K-D from the hot water bath.

10.7 Momentarily remove the Snyder column, add 50 mL hexane and a new boiling chip and replace the column. Raise the temperature of the water bath to 85 to 90°C. Concentrate the extract as in Section 10.6, except using hexane to prewet the column. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. Stopper the concentrator tube and store refrigerated if further processing will not be performed immediately.

10.8 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

10.9 Unless the sample is known to require cleanup, proceed to analysis by gas chromatography.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of each compound of interest is no less than 85%.

11.2 Florisil column cleanup for chlorinated hydrocarbons.

11.2.1 Adjust the sample extract to 10 mL with hexane.

11.2.2 Place a 12-g charge of activated Florisil in a 10-mm ID chromatography column. After settling the Florisil by tapping the column, add a 1 to 2 cm layer of anhydrous granular sodium sulfate to the top. Allow to cool, then pre-elute the column, with 100 mL of petroleum ether. Discard the eluate and just prior to exposure of the sodium sulfate layer to air, quantitatively transfer the sample extract into the column by decantation and subsequent petroleum ether washings. Discard the eluate. Just prior to exposure of the sodium sulfate layer to the air, begin eluting the column with 200 mL petroleum ether and collect the eluate in a 500-mL K-D flask equipped with a 10-mL concentrator tube. This fraction should contain all of the chlorinated hydrocarbons.

11.2.3 Concentrate the fraction by K-D as in section 10.6 except prewet the column with hexane. When the apparatus is cool, remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. Analyze by GC.

12. Gas Chromatography

12.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. This table includes retention times and MDL that were obtained under these conditions. Examples of the parameter separations achieved by this column are shown in Figures 1 and 2. Other packed columns, chromatographic conditions, or detectors may be used if the requirements of Section 8.2 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 are met.

12.2 Calibrate the system daily as described in Section 7.

12.3 If the internal standard approach is being used, the internal standard must be added to the sample extract and mixed thoroughly, immediately before injection into the instrument.

12.4 Inject 2 to 5 μL of the sample extract using the solvent-flush technique[9]. Smaller (1.0 μL) volumes can be injected if automatic devices are employed. Record the volume of the extract to the nearest 0.1 mL, the volume injected to the nearest 0.05 μL, and the resulting peak size in area or peak height units.

12.5 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time for a compound can be used to calculate a suggested window size; however, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

12.6 If the response for the peak exceeds the working range of the system, dilute the extract and reanalyze.

12.7 If the measurement of the peak response is prevented by the presence of interferences, further cleanup is required.

13. Calculations

13.1 Determine the concentration of individual compounds in the sample.

13.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the peak response using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from Equation 2:

$$\text{Eq. 2. Concentration}, \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.
V_i = Volume of extract injected (μL).
V_t = Volume of total extract (μL).
V_s = Volume of water extracted (mL).

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and Equation 3.

$$\text{Eq. 3. Concentration}, \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Response for the parameter to be measured.
A_{is} = Response for the internal standard.
I_s = Amount of internal standard added to each extract (μg).
V_o = Volume of water extracted, in liters.

13.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in section 8.4, data for the affected parameters must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(10). Similar results were achieved using representative wastewaters.

14.2 This method has been tested for linearity of spike recovery from reagent water and has been demonstrated to be applicable over the concentration range from the 4 x MDL up to 1000 x MDL(10).

14.3 In a single laboratory (EMSL-Cincinnati), using three wastewaters spiked at six concentration levels, the average recoveries presented in Table 2 were obtained. The standard deviation of the percent recovery is also included in Table 2.

14.4 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. "Determination of Chlorinated Hydrocarbons In Industrial and Municipal Wastewaters." Report for EPA Contract 68-03-2625 (In preparation).
3. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, 1980.
4. "Carcinogens - Working With Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
5. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
7. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
8. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.
9. Burke, J. A. "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).
10. "Development of Detection Limits, EPA Method 612, Chlorinated Hydrocarbons," Special letter report for EPA Contract 68-03-2625, Environmental Monitoring and Support Laboratory - Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limits*

Parameter	Retention Time (min.)	Method Detection Limit (μg/L)
1,3-Dichlorobenzene	6.8	1.19
1,4-Dichlorobenzene	7.6	1.34
Hexachloroethane	8.3	0.03
1,2-Dichlorobenzene	9.3	1.14
Hexachlorobutadiene	20.0	0.34
1,2,4-Trichlorobenzene	22.3	0.05
2-Chloronaphthalene	3.6[a]	0.94
Hexachlorobenzene	10.1[a]	0.05

Column conditions: Supelcoport (80/100 mesh) coated with 1.5% OV-1/2.4% OV-225 packed in a 1.8 m x 2 mm ID glass column with 5% Methane/95% Argon carrier gas at a flow rate of 25 mL/min. Column temperature, isothermal at 75°C, except as other wise indicated.

[a] *- Column temperature 165°C.*

Table 2. *Single Operator Accuracy and Precision*

Parameter	Average Percent Recovery	Standard Deviation %	Spike Range (μg/L)	Number of Analyses	Matrix Types
2-Chloronaphthalene	76	25	19.1-268	18	3
1,2-Dichlorobenzene	82	10	29.8-356	18	3
1,3-Dichlorobenzene	86	18	20.4-238	18	3
1,4-Dichlorobenzene	89	20	23.0-324	18	3
Hexachlorobenzene	95	12	1.29-14.9	18	3
Hexachlorobutadiene	96	10	3.12-36.8	18	3
Hexachloroethane	99	12	1.02-14.8	18	3
1,2,4-Trichlorobenzene	96	16	15.1-216	18	3

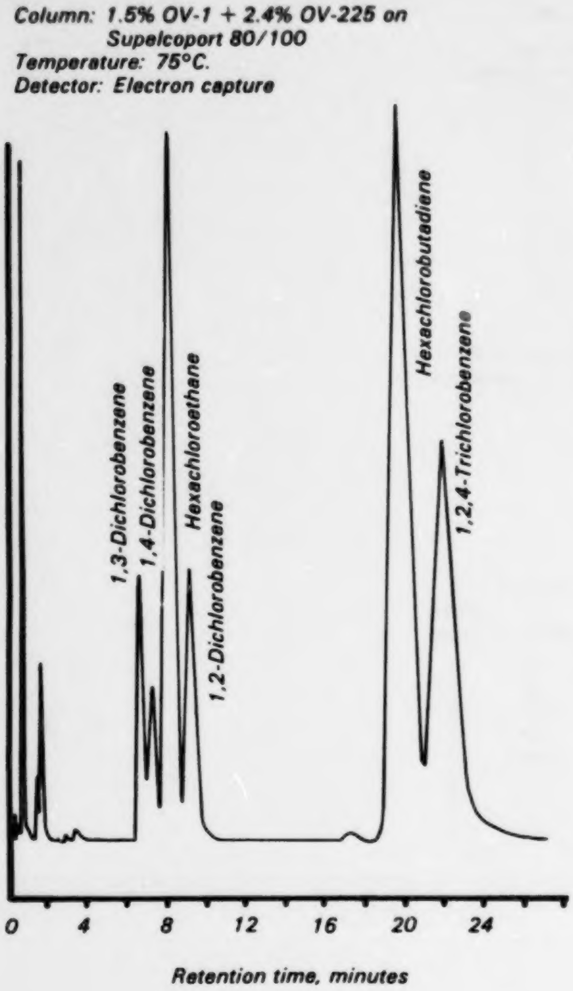


Figure 1. *Gas chromatogram of chlorinated hydrocarbons.*

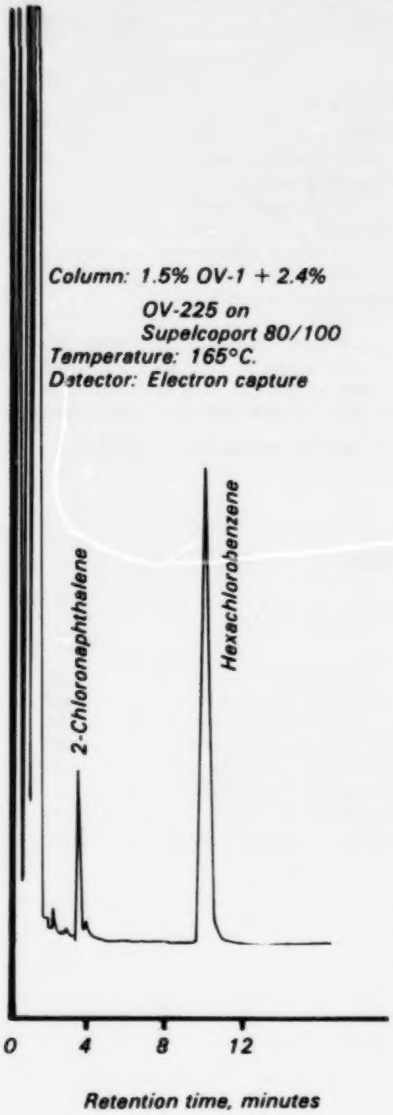


Figure 2. *Gas chromatogram of chlorinated hydrocarbons.*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

2,3,7,8-Tetrachlorodibenzo-p-Dioxin — Method 613

1. Scope and Application

1.1 This method covers the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). The following parameter may be determined by this method:

Parameter	STORET No.	CAS No.
2,3,7,8-TCDD	34675	1746-01-6

1.2 This is a gas chromatographic/mass spectrometer (GC/MS) method applicable to the determination of 2,3,7,8-TCDD in municipal and industrial discharges as provided under 40 CFR 136.1. Method 625 may be used to screen samples for 2,3,7,8-TCDD. When the screening test is positive, the final qualitative confirmation and quantification must be made using method 613.

1.3 The method detection limit (MDL, defined in Section 14.1)(1) for 2,3,7,8-TCDD is listed in Table 1. The MDL for a specific wastewater may be different depending upon the nature of interferences in the sample matrix.

1.4 Because of the extreme toxicity of this compound, the analyst must prevent exposure to himself, or to others, by materials known or believed to contain 2,3,7,8-TCDD. Section 4 of this method contains guidelines and protocols that serve as minimum safe-handling standards in a limited access laboratory.

1.5 Any modification of this method, beyond those expressly permitted, shall be considered as major modifications subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.6 This method is restricted to use only by or under the supervision of analysts experienced in the use of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A one-liter sample of wastewater is spiked with an internal standard of labeled 2,3,7,8-TCDD. The spiked sample is then extracted with methylene chloride using separatory funnel techniques. The extract is concentrated and exchanged to hexane while being concentrated to a volume of 1.0 mL or less. Capillary column GC/MS conditions are described which allow for the separation and measurement of 2,3,7,8-TCDD in the extract(2,3).

2.2 The method provides selected column chromatographic cleanup procedures to aid in the elimination of interferences that may be encountered.

3. Interferences

3.1 · Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample

118<

processing hardware that lead to discrete artifacts and/or elevated backgrounds at the ions monitored. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned.(4) Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and distilled water. Glassware should then be drained dry, and heated in a muffle furnace at 400 °C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store it inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled. 2,3,7,8-TCDD is often associated with other interfering chlorinated compounds which are at concentrations several magnitudes higher than that of 2,3,7,8-TCDD. The cleanup procedures in Section 11 can be used to overcome many of these interferences, but unique samples may require additional cleanup approaches(1,5-7) to eliminate false positives and achieve the method detection limit listed in Table 1.

3.3 The primary column, SILAR-10C, resolves 2,3,7,8-TCDD from the other 21 isomers(3). Positive results obtained using any other gas chromatographic column must be confirmed using this column.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are identified(8-10). Benzene and 2,3,7,8-TCDD have been identified as suspected human or mammalian carcinogens.

4.2 Each laboratory must develop a strict safety program for handling of 2,3,7,8-TCDD. The following laboratory practices are recommended:

4.2.1 Contamination of the laboratory will be minimized by conducting all manipulations in a hood.

4.2.2 The effluents of sample splitters for the gas chromatograph and roughing pumps on the GC/MS should pass through either a column of activated charcoal or be bubbled through a trap containing oil or high-boiling alcohols.

4.2.3 Liquid waste should be dissolved in methanol or ethanol and irradiated with ultraviolet light with wavelength greater than 290 nm for several days. (Use F 40 BL lamps or equivalent.) Analyze liquid wastes and dispose of the solutions when 2,3,7,8-TCDD can no longer be detected.

4.3 Dow Chemical U.S.A. has issued the following precautions (revised 11/78) for safe handling of 2,3,7,8-TCDD in the laboratory:

4.3.1 The following statements on safe handling are as complete as possible on the basis of available toxicological information. The precautions for safe handling and use are necessarily general in nature since detailed, specific recommendations can be made only for the particular exposure and circumstances of each individual use. Inquiries about specific operations or uses may be addressed to the Dow Chemical Company. Assistance in evaluating the health hazards of particular plant conditions may be obtained from certain consulting laboratories and from State Departments of Health or of Labor, many of which have an industrial health service. 2,3,7,8-TCDD is extremely toxic to laboratory animals. However, it has been handled for years without injury in analytical and biological laboratories. Techniques used in handling radioactive and infectious materials are applicable to 2,3,7,8-TCDD.

4.3.1.1 Protective Equipment: Throw-away plastic gloves, apron or lab coat, safety glasses and lab hood adequate for radioactive work.

4.3.1.2 Training: Workers must be trained in the proper method of removing of contaminated gloves and clothing without contacting the exterior surfaces.

4.3.1.3 Personal Hygiene: Thorough washing of hands and forearms after each manipulation and before breaks (coffee, lunch, and shift).

4.3.1.4 Confinement: Isolated work area, posted with signs, segregated glassware and tools, plastic-backed absorbent paper on benchtops.

4.3.1.5 Waste: Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors must be trained in safe handling of waste.

4.3.1.6 Disposal of Wastes: 2,3,7,8-TCDD decomposes above 800 °C. Low-level waste such as the absorbent paper, tissues, animal remains and plastic glvoes may be burned in a good incinerator. Gross quantities (milligrams) should be packaged securely and disposed through commercial or governmental channels which are capable of handling high-level radioactive wastes or extremely toxic wastes. Liquids should be allowed to evaporate in a good hood and in a disposable container. Residues may then be handled as above.

4.3.1.7 Decontamination: Personal—any mild soap with plenty of scrubbing action: Glassware, Tools, and Surfaces—Chlorothene NU Solvent (Trademark of the Dow Chemical Company) is the least toxic solvent shown to be effective. Satisfactory cleaning may be accomplished by rinsing with Chlorothene, then washing with any detergent and water. Dish water may be disposed to the sewer. It is prudent to minimize solvent wastes because they may require special disposal through commercial sources which are expensive.

4.3.1.8 Laundry: Clothing known to be contaminated should be disposed with the precautions described under "Disposal of Wastes." Lab coats or other clothing worn in 2,3,7,8-TCDD work area may be laundered. Clothing should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows the problem. The washer should be run through a cycle before being used again for other clothing.

4.3.1.9 Wipe Tests: A useful method of determining cleanliness of work surfaces and tool is to wipe the surface with a piece of filter paper. Extraction and analysis by gas chromatography can achieve a limit of sensitivity of 0.1 μg per wipe. Less than 1 μg 2,3,7,8-TCDD per sample indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 μg on a wipe sample indicates an acute hazard and requires prompt cleaning before further use of the equipment or work space and indicates further that unacceptable work practices have been employed in the past.

4.3.1.10 Inhalation: Any procedure that may produce airborne contamination must be done with good ventilation. Gross losses to a ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in case of an accident.

4.3.1.11 Accidents: Remove contaminated clothing immediately, taking precautions not to contaminate skin or other articles. Wash exposed skin vigorously and repeatedly until medical attention is obtained.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4 °C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.1.3 Clearly label all samples as "POISON" and ship according to U.S.D.O.T. requirements.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel—2000-mL and 125-mL, with Teflon stopcock.

5.2.2 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.3 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.4 Snyder column, Kuderna-Danish—three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.5 Snyder column, Kuderna-Danish—two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.6 Vials—Amber glass, 10- to 15-mL capacity, with Teflon-lined screw cap.

5.2.7 Chromatography column—300 mm long × 10 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.2.8 Chromatography column—400 mm long × 11 mm ID with coarse fritted disc at bottom and Teflon stopcock.

5.3 Boiling chips—approximately 10/40 mesh. Heat to 400 °C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (± 2 °C). The bath should be used in a hood.

5.5 GC/MS system.

5.5.1 Gas chromatograph—An analytical system complete with all required accessories including syringes, analytical columns, and gases. The injection port must be designed for capillary columns. Either split, splitless, or on-column injection techniques may be employed, as long as the requirements of Section 7.1.1 are achieved.

5.5.2 Primary column—50 m long × 0.25 mm ID glass, coated with SILAR-10C (or equivalent). An equivalent column must resolve 2,3,7,8-TCDD from the other 21 TCDD isomers. Guidelines for the use of alternate columns are provided in Section 12.1.

5.5.3 Mass Spectrometer—Either low resolution mass spectrometers (LRMS) or high resolution mass spectrometers (HRMS) may be used. The mass spectrometer must be equipped with a 70 volt (nominal) ion source and be capable of acquiring ion abundance data in real time Selected Ion Monitoring (SIM) for groups of four or more ions.

5.5.4 GC/MS interface—Any gas chromatograph to mass spectrometer interface can be used that achieves the requirements of Section 7.1.1 constructed of glass or glass-lined materials are recommended. Glass surfaces can be deactivated by silanizing with dichlorodimethylsilane. To achieve maximum sensitivity, the exit end of the capillary column should be placed in the ion source. A short piece of fused silica capillary can be used as the interface to overcome problems associated with straightening the exit end of glass capillary columns.

5.5.5 The SIM data acquired during the chromatographic program is defined as the Selected Ion Current Profile (SICP). The SICP can be acquired under computer control or as real time analog output. If computer control is used, there must be software available to plot the SICP and report peak height or area data for any ion in the SICP between specified time or scan number limits.

5.6 Balance—Analytical, capable of accurately weighing 0.0001g.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of 2,3,7,8-TCDD.

6.2 Sodium hydroxide solution—(ACS) Dissolve 400 g NaOH in reagent water and dilute to one liter. Wash the solution with methylene chloride and with hexane before use.

6.3 Sodium thiosulfate—(ACS) Granular.

6.4 Sulfuric acid (Conc.)—(ACS) sp. gr. 1.84.

6.5 Methylene chloride, hexane, benzene, tetradecane—Pesticide quality or equivalent.

6.6 Sodium sulfate—(ACS) Granular, anhydrous (purified by heating at 400 °C for four hours in a shallow tray).

6.7 Alumina—neutral, 80/200 mesh (Fisher Scientific Co., No. A-540 or equivalent). Before use, activate for 24 hours at 130 °C in a foil covered glass container.

6.8 Silica gel—high purity grade, 100/120 mesh, (Fisher Scientific Co., No. S-679 or equivalent).

6.9 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions. Acetone should be used as the solvent for spiking solutions; ortho-xylene is recommended for calibration standards for split injectors; and tetradecane is recommended for splitless or on-column injectors. Analyze stock internal standards to verify the absence of native 2,3,7,8-TCDD.

6.9.1 Prepare stock standard solutions of 2,3,7,8-TCDD (mol wt 320) and either $^{37}Cl_4$ 2,3,7,8-TCDD (mol wt 328) or $^{13}C_{12}$ 2,3,7,8-TCDD (mol wt 332) in an isolated area by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality solvent, dilute to volume in a 10-mL volumetric flask. If compound purity is certified at 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards can be used at any concentration if they are certified by the manufacturer or by an independent source.

6.9.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store in an isolated refrigerator protected from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards or spiking solutions from them.

6.9.3 Stock standard solutions must be replaced after six months or sooner if comparison with check standards indicates a problem.

6.10 Internal standard spiking solutions (25 ng/mL)—Using stock standard solution. Prepare a spiking solution in acetone of either $^{13}C_{12}$ or $^{37}Cl_4$ 2,3,7,8-TCDD at a concentration of 25 ng/mL. (See Section 10.2.1.)

7. Calibration

7.1 Establish gas chromatographic conditions for the GC/MS system equivalent to those indicated in Table 1 and SIM conditions for the mass spectrometer as described in Section 12.2. The GC/MS system must be calibrated using the internal standard technique. By injecting calibration standards, establish ion response factors for 2,3,7,8-TCDD vs. an internal standard (either $^{13}C_{12}$ or $^{37}Cl_4$ 2,3,7,8-TCDD).

7.1.1 Using stock standards, prepare GC/MS calibration standard solutions that will allow measurement of relative response factors of at least three concentration ratios of 2,3,7,8-TCDD to internal standard. Each solution must be prepared to contain the internal standard at a concentration of 25 ng/mL. If any interferences are contributed by the internal standard at m/e 320 and 322, its concentration may be reduced in calibration standards and in the internal spiking solution (Section 6.10). One of the calibration standard solutions should be prepared to contain 2,3,7,8-TCDD representing a concentration near, but above, the MDL. The other 2,3,7,8-TCDD concentrations should correspond to the expected range of concentrations found in real samples.

7.1.2 Using injections of 2 to 5 µL, tabulate peak height or area response against the concentration of 2,3,7,8-TCDD and internal standard in each calibration standard, and calculate response factors (RF) for 2,3,7,8-TCDD using equation 1.

$$\text{Eq. 1}\quad RF = (A_s C_{is})/(A_{is} C_s)$$

where:

A_s = SIM response for 2,3,7,8-TCDD m/e 320.

A_{is} = SIM response for internal standard, m/e 332 for $^{13}C_{12}$ 2,3,7,8-TCDD, m/e 328 for $^{37}Cl_4$ 2,3,7,8-TCDD.

C_{is} = Concentration of the internal standard, (µg/L).

C_s = Concentration of 2,3,7,8-TCDD (µg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.1.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more 2,3,7,8-TCDD calibration standards. If the response for 2,3,7,8-TCDD varies from the predicted response by more than ± 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

7.2 Before using any cleanup procedure, the analyst must process a series of calibration standards through the procedure to validate elution patterns and the absence of interferences from the reagents.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in both mass spectrometry and gas chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike and analyze a minimum of 10% of all samples with native 2,3,7,8-TCDD to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Using stock standard solution, prepare a quality control check sample concentrate containing 2,3,7,8-TCDD at a concentration of 15 ng/mL in acetone.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for the results. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 2, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > 2p$ or $|X - R| > 2p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the result of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria and define the performance of the laboratory for each spike concentration and parameter being measured.

8.3.1 Calculate upper and lower control limits for method performance:

Upper Control Limit (UCL) = $R + 3s$
Lower Control Limit (LCL) = $R - 3s$

where R and s are calculated as in Section 8.2.3. The UCL and LCL can be used to construct control charts(11) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 The laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(10).

8.4 The laboratory is required to collect a portion of their samples in duplicate to monitor spike recoveries. The frequency of spiked sample analysis must be at least 10% of all samples or one sample per month, whichever is greater. One aliquot of the sample must be spiked and analyzed as described in Section 8.2. If the recovery for 2,3,7,8-TCDD does not fall within the control limits for method performance, the results reported for 2,3,7,8-TCDD in all samples processed as part of the same set must be qualified as described in Section 13.4. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(12) should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4 °C and protected from light from the time of collection until extraction. If the sample contains residual chlorine, add 80 mg of sodium thiosulfate per each liter of sample. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used for measurement of residual chlorine(13). Field test kits are available for this purpose.

9.3 Label all samples and containers POISON and ship according to applicable U.S. Department of Transportation regulations.

9.4 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction(2).

10. Sample Extraction

CAUTION: When using this method to analyze for 2,3,7,8-TCDD, all of the following operations must be performed in a limited access laboratory with the analyst wearing full protective covering for all exposed skin surfaces. See Section 4.2.

10.1 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel.

10.2 Add 1.00 mL of internal standard spiking solution to the sample in the separatory funnel. If the final extract will be concentrated to a fixed volume below 1.00 mL (Section 12.3), only that volume of spiking solution should be added to the sample so that the final extract will represent 25 ng/mL at the time of analysis.

10.3 Add 60 mL methylene chloride to the sample bottle, seal, and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-mL Erlenmeyer flask.

10.4 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner.

10.5 Assemble a Kuderna-Danish (K-D) concentrator by attaching a

10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.6 Pour the combined extract into the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.7 Add one or two clean boiling chips to the evaporative flask and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top. Place the K-D apparatus on a hot water bath (60 to 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes.

10.8 Momentarily remove the Snyder column, add 50 mL hexane and a new boiling chip and replace the column. Raise the temperature of the water bath to 85 to 90 °C. Concentrate the extract as in Section 10.7, except using hexane to prewet the column. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of hexane. A 5-mL syringe is recommended for this operation. Set aside a K-D glassware for reuse in Section 10.14.

10.9 Pour the hexane from the concentrator tube into a 125-mL separatory funnel. Rinse the concentrator tube four times with 10-mL aliquots of hexane. Combine all rinses in the 125-mL separatory funnel.

10.10 Add 50 mL of 10N sodium hydroxide solution to the funnel and shake for 30 to 60 seconds. Discard the aqueous phase.

10.11 Perform a second wash of the organic layer with 50 mL of reagent water. Discard the aqueous phase.

10.12 Wash the hexane layer with at least two 50-mL aliquots of concentrated sulfuric acid. Continue washing the hexane layer with 50-mL aliquots of concentrated sulfuric acid until the acid layer remains colorless. Discard all acid fractions.

10.13 Wash the hexane layer with two 50-mL aliquots of reagent water. Discard the aqueous phases.

10.14 Transfer the hexane layer into a 125-mL Erlenmeyer flask containing 1 to 2 g anhydrous sodium sulfate. Swirl the flask for 30 seconds and decant the hexane into the reassembled K-D apparatus. Complete the quantitative transfer with two 10-mL hexane rinses of the Erlenmeyer flask.

10.15 Replace the one or two clean boiling chips and concentrate the extract to 6 to 10 mL as described in Section 10.8.

10.16 Add a clean boiling chip and attach a micro-Snyder column. Prewet the column by adding about 1 mL hexane to the top. Place the K-D apparatus on the 80 °C water bath so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apparatus and allow it to drain for at least 10 minutes while cooling. Remove the micro Snyder column and rinse its lower joint into the concentrator tube with 0.2 mL hexane. Adjust the extract volume to 1.0 mL with hexane. Stopper the concentrator tube and store refrigerated and protected from light if GC/MS analysis or cleanup will not be performed immediately.

10.17 Determine the original sample volume by refilling the sample bottle to the mark with water and measuring the volume in a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Cleanup and Separation

11.1 Cleanup procedures may not be necessary for a relatively clean sample matrix. The cleanup procedures recommended in this method have been used for the analysis of various clean waters and industrial effluents. The single operator precision and accuracy data in Table 2 was gathered using the recommended cleanup procedures. If particular circumstances demand the use of an alternative cleanup procedure, the analyst must determine the elution profile and demonstrate that the recovery of 2,3,7,8-TCDD is reproducible and equivalent to the recovery of the internal standard. Two cleanup column options are offered to the analyst in this section. The alumina column should be used first to overcome interferences. If background problems are still encountered, the silica gel column may be helpful. Other cleanup procedures have been described to overcome special interference problems(1,5-7).

11.2 Alumina column cleanup for 2,3,7,8-TCDD.

11.2.1 Fill a 300 mm long × 10 mm ID chromatography column with activated alumina to the 150 mm level, tapping the column gently to settle the alumina. Add 10 mm anhydrous sodium sulfate to the top of the alumina.

11.2.2 Preelute the column with 50 mL hexane. Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the entire 1.0 mL sample extract onto the column, using two additional 2-mL portions of hexane to complete the transfer.

11.2.3 Just prior to exposure of the sodium sulfate layer to the air, add 50 mL 3% methylene chloride/97% hexane (V/V) and continue the elution of the column. Discard the eluate.

11.2.4 Next elute the column with 50 mL 20% methylene chloride/80% hexane (V/V) into a 500 mL K-D flask equipped with a 10-mL concentrator tube. Concentrate the collected fraction to 1.0 mL by standard K-D techniques and analyze by GC/MS.

11.3 Silica gel column cleanup for 2,3,7,8-TCDD.

11.3.1 Fill a 400 mm long × 11 mm ID chromatography column with silica gel to the 300 mm level, tapping the column gently to settle the silica gel. Add 10 mm anhydrous sodium sulfate to the top of the silica gel.

11.3.2 Preelute the column with 50 mL 20% benzene/80% hexane (V/V). Adjust the elution rate to 1 mL/min. Discard the eluate and just prior to exposure of the sodium sulfate layer to the air, transfer the entire 1.0 mL sample extract onto the column, using two 2 mL portions of 20% benzene/80% hexane to complete the transfer.

11.3.3 Just prior to exposure of the sodium sulfate layer to the air, add 40 mL 20% benzene/80% hexane to the column. Collect the eluate in a clean

500-mL K-D flask equipped with a 10-mL concentrator tube.

11.3.4 Evaporate the fraction to 1.0 mL by standard K-D techniques and analyze by GC/MS.

12. GC/MS Analysis

12.1 Table 1 summarizes the recommended gas chromatographic capillary column and operating conditions for the instrument. Included in this table are the estimated retention time and MDL that can be achieved by this method. Other capillary columns or chromatographic conditions may be used to screen samples if the requirements of Section 8.2 are met. Confirmation of 2,3,7,8-TCDD must be accomplished using a GC column that separates 2,3,7,8-TCDD from all other TCDD isomers.

12.2 Analyze standards and samples with the mass spectrometer operating in the SIM mode using a dwell time to give at least seven points per peak. For LRMS, use ions at m/e 320, 322, and 257 for 2,3,7,8-TCDD and either the ion at m/e 328 for ^{37}Cl 2,3,7,8-TCDD or m/e 332 for ^{13}C 2,3,7,8-TCDD. For HRMS, use ions at m/e 319.8965 and 321.8936 for 2,3,7,8-TCDD and either the ion at m/e 327.8847 for ^{37}Cl 2,3,7,8-TCDD or m/e 331.9367 for ^{13}C 2,3,7,8-TCDD.

12.3 If lower detection limits are required, the extract may be carefully evaporated to dryness under a gentle stream of nitrogen with the concentrator tube in a water bath at about 40 °C. Do this immediately before GC/MS analysis. Redissolve the extract in the desired final volume of ortho-xylene or tetradecane. The method performance data reported in Section 14 was gathered using a final extract volume of 0.2 mL.

12.4 Calibrate the system daily as described in Section 7.1.3. The volume of calibration standard injected must be measured, or be the same as all sample injection volumes.

12.5 Inject a 2 to 5 µL aliquot of the sample extract.

12.6 The presence of 2,3,7,8-TCDD is qualitatively confirmed if all of the following criteria are achieved.

12.6.1 The gas chromatographic column must resolve 2,3,7,8-TCDD from the other 21 TCDD isomers.

12.6.2 The ions for native 2,3,7,8-TCDD (LRMS-m/e 320, 322, and 257 and HRMS-m/e 320 and 322) and labeled 2,3,7,8-TCDD (m/e 328 or 332) must exhibit a simultaneous maximum at a retention time that matches that of native 2,3,7,8-TCDD in the calibration standard, within the performance specifications of the analytical system.

12.6.3 The chlorine isotope ratio at m/e 320 and m/e 322 must agree to within ±10% of that in the calibration standard.

12.6.4 The signal of all peaks must be greater than 2.5 times the noise level.

12.7 For quantitation, measure the response of the m/e 320 peak for 2,3,7,8-TCDD and the m/e 332 peak for $^{13}C_{12}$ 2,3,7,8-TCDD or the m/e 328 peak for $^{37}Cl_4$ 2,3,7,8-TCDD.

12.8 Co-eluting impurities are suspected if all criteria are achieved except those in Section 12.6.3. In this case, another SIM analysis using ions at m/e 257, 259, 320 and either m/e 328 or m/e 322 can be performed. The ions at m/e 257 and m/e 259 are indicative of the loss of one chlorine and one carbonyl group from 2,3,7,8-TCDD. If the ions m/e 257 and m/e 259 give a chlorine isotope ratio that agrees to within ±10% of the same cluster in the calibration standards, than the presence of TCDD can be confirmed. Co-eluting DDD, DDE, and PCB residues can be confirmed, but will require another injection using the appropriate SIM ions or full repetitive mass scans. If the response for ^{37}Cl 2,3,7,8-TCDD at m/e 328 is too large, PCB contamination is suspected and can be confirmed by examining the response at both m/e 326 and m/e 328. The ^{37}Cl 2,3,7,8-TCDD internal standard gives negligible response at m/e 326. These pesticide residues can be removed using the alumina column cleanup.

12.9 If broad background interference restricts the sensitivity of the GC/MS analysis, the analyst should employ additional cleanup procedures and reanalyze by GC/MS.

12.10 In those circumstances where these procedures do not yield a definitive conclusion, then the use of high resolution mass spectrometry is suggested.[5]

13. Calculations

13.1 Calculate the concentration of 2,3,7,8-TCDD in the sample using the response factor (RF) determined in 7.1.2 and equation 2.

Eq. 2

$$\text{Concentration, } \mu g/l = \frac{(A_{is})(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = SIM response for 2,3,7,8-TCDD ion at m/e 320.

A_{is} = SIM response for the internal standard ion at m/e 328 or 332.

I_s = Amount of internal standard added to each extract (µg).

V_o = Volume of water extracted, in liters.

13.2 For each sample, calculate the percent recovery of the internal standard by comparing the area of the ion peak measured in the sample to the area of the same peak in the calibration standard. If the recovery is below 50%, the results of the sample analysis must be qualified as described in Section 13.4.

13.3 Report results in micrograms per liter. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.4 For samples processed as part of a set where the laboratory spiked sample recovery falls outside of the control limits in Section 8.3 or the internal standard recovery is below 50%, the data for 2,3,7,8-TCDD must be labeled as suspect.

14. Method Performance

14.1 Method detection limit—The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentration listed in Table 1 was obtained using reagent water(14).

14.2 In a single laboratory (Monsanto Research Corporation), using spiked samples, the average recovery presented in Table 2 was obtained.(14) The average standard deviation of the percent recovery is also included in Table 2. The results for quality control checks for this study are presented in Table 3.

14.3 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.

2. "Determination of 2,3,7,8-TCDD in Industrial and Municipal Wastewaters." Report for EPA Control 68-03-2635 (In preparation).
3. Buser, H.R., and Rappe, C. "High Resolution Gas Chromatography of the 22 Tetrachlorodibenzo-p-dioxin Isomers," *Analytical Chemistry, 52,* 2257, (1980).
4. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Test and Materials, Philadelphia, PA, p. 679, (1980).
5. Harless, R.L., E.O. Oswald, and M.K. Wilkinson, "Sample Preparation and Gas Chromatography/Mass Spectrometry Determination of 2,3,7,8-Tetrachlorodibenzo-p-dioxin." *Analytical Chemistry, 52,* 1239 (1980).
6. Lamparski, L.L. and T.J. Nestrick. "Determination of Tetra-, Hepta-, and Octachlorodibenzo-p-dioxin Isomers in Particulate Samples at Parts per Trillion Levels," *Analytical Chemistry, 52,* 2045 (1980).
7. Longhorst, M.L. and L.A. Shadoff, "Determination of Parts-per-Trillion Concentrations of Tetra-, Hexa-, and Octachlorodibenzo-p-dioxins in Human Milk," *Analytical Chemistry, 52,* 2037, (1980).
8. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
9. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
10. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
11. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
12. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA 1980.
13. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
14. "Determination of Method Detection Limits for EPA Method 613," Special letter report for EPA Contract 68-03-2863, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

Table 1. *Chromatographic Conditions and Method Detection Limit*

Parameter	*Retention Time (min.)*	*Detection Limit (g/L)*
2,3,7,8-TCDD	*34.5*	*0.002*

Column conditions: SILAR-10C coated on a 50 m long 0.25 mm ID glass column with helium carrier gas at 30 cm/sec linear velocity, splitless injector. Column temperature programmed: isothermal, 100°C for 3 minutes, then programmed at 20°C/min to 180°C, and 2°C/min to 250°C.

Table 2. *Single Operator Accuracy and Precision*

Parameter	*Matrix*	*Spike µg/L*	*Average Percent Recovery*	*Standard Deviation %*
2,3,7,8-TCDD	*Reagent water*	*0.005*	*95.4*	*10.2*
2,3,7,8-TCDD	*Industrial waste*	*0.005*	*85.8*	*6.6*
2,3,7,8-TCDD	*Municipal waste*	*0.025*	*92.4*	*18.7*

Table 3. *Quality Control Results (14)*

Matrix	*320/322 Isotope Ratios*	*Internal Standard Recovery*
Reagent water	*0.79 ± 0.04*	*87%*
Industrial waste	*0.81 ± 0.01*	*104%*
Municipal waste	*0.83 ± 0.02*	*14%*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

Test Method

Purgeables — Method 624

1. Scope and Application

1.1 This method covers the determination of a number of purgeable organics. The following parameters may be determined by this method:

Parameter	STORET No.	CAS No.
Benzene	34030	71-43-2
Bromodichloromethane	32101	75-27-4
Bromoform	32104	75-25-2
Bromomethane	34413	74-83-9
Carbon tetrachloride	32102	56-23-5
Chlorobenzene	34301	108-90-7
Chloroethane	34311	75-00-3
2-Chloroethylvinyl ether	34576	110-75-8
Chloroform	32106	67-66-3
Chloromethane	34418	74-87-3
Dibromochloromethane	32105	124-48-1
1,2-Dichlorobenzene	34536	95-50-1
1,3-Dichlorobenzene	34566	541-73-1
1,4-Dichlorobenzene	34571	106-46-7
1,1-Dichloroethane	34496	75-34-3
1,2-Dichloroethane	34531	107-06-2
1,1-Dichloroethene	34501	75-35-4
trans-1,2-Dichloroethene	34546	156-60-5
1,2-Dichloropropane	34541	78-87-5
cis-1,3-Dichloropropene	34704	10061-01-5
trans-1,3-Dichloropropene	34699	10061-02-6
Ethyl benzene	34371	100-41-4
Methylene chloride	34423	75-09-2
1,1,2,2-Tetrachloroethane	34516	79-34-5
Tetrachloroethene	34475	127-18-4
Toluene	34010	108-88-3
1,1,1-Trichloroethane	34506	71-55-6
1,1,2-Trichloroethane	34511	79-00-5
Trichloroethene	39180	79-01-6
Trichlorofluoromethane	34488	75-69-4
Vinyl chloride	39175	75-01-4

1.2 The method may be extended to screen samples for acrolein (STORET No. 34210, CAS No. 107-02-8) and acrylonitrile (STORET 34215, CAS No. 107-13-1), however, the preferred method for these two compounds is method 603.

1.3 This is a purge and trap gas chromatographic/mass spectrometer

(GC/MS) method applicable to the determination of the compounds listed above in municipal and industrial discharges as provided under 40 CFR 136.1.

1.4 The method detection limit (MDL, defined in Section 14.1)(1) for each parameter is listed in Table 1. The MDL for a specific wastewater differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 Until the U.S. Environmental Protection Agency establishes performance criteria based upon the results of interlaboratory testing, any alternative GC/MS method which meets the performance criteria described in Section 8.2 will be permitted. Performance must be verified for such modification by analyzing wastewater as described in Section 8.2.2. In addition, the laboratory must successfully participate in the applicable performance evaluation studies.

1.6 This method is restricted to use by or under the supervision of analysts experienced in the use of purge and trap systems and gas chromatograph/ mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 An inert gas is bubbled through a 5-mL sample contained in a specially-designed purging chamber at ambient temperature. The purgeables are efficiently transferred from the aqueous phase to the vapor phase. The vapor is swept through a sorbent column where the purgeables are trapped. After purging is completed, the sorbent column is heated and backflushed with the inert gas to desorb the purgeables onto a gas chromatographic column. The gas chromatograph is temperature programmed to separate the purgeables which are then detected with a mass spectrometer(2,3).

3. Interferences

3.1 Impurities in the purge gas, organic compounds out-gassing from the plumbing ahead of the trap and solvent vapors in the laboratory account for the majority of contamination problems. The analytical system must be demonstrated to be free from contamination under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5. The use of non-TFE plastic tubing, non-TFE thread sealants, or flow controllers with rubber components in the purging device should be avoided.

3.2 Samples can be contaminated by diffusion of volatile organics (particularly fluorocarbons and methylene chloride) through the septum seal into the sample during shipment and storage. A field reagent blank prepared from reagent water and carried through the sampling and handling protocol can serve as a check on such contamination.

3.3 Contamination by carry over can occur whenever high level and low level samples are sequentially analyzed. To reduce carry over, the purging device and sample syringe must be rinsed with reagent water between sample analyses. Whenever an unusually concentrated sample is encountered, it should be followed by an analysis of reagent water to check for cross contamination. For samples containing large amounts of water-soluble materials, suspended solids, high boiling compounds or high purgeable levels, it may be necessary to wash out the purging device with a detergent solution, rinse it with distilled water, and then dry it in a 105 °C oven between analyses. The trap and other parts of the system are also subject to contamination; therefore, frequent bakeout and purging of the entire system may be required.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified(5-7) for the information of the analyst.

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens: benzene, carbon tetrachloride, chloroform, 1,4-dichlorobenzene, and vinyl chloride. Primary standards of these toxic compounds should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be worn when the analyst handles high concentrations of these toxic compounds.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete sampling.

5.1.1 Vial—25-mL capacity or larger, equipped with a screw cap with hole in center (Pierce #13075 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105 °C before use.

5.1.2 Septum—Teflon-faced silicone (Pierce #12722 or equivalent). Detergent wash, rinse with tap and distilled water, and dry at 105 °C for one hour before use.

5.2 Purge and trap device—The purge and trap device consists of three separate pieces of equipment: the sample purger, trap, and the desorber. Several complete devices are now commercially available.

5.2.1 The sample purger must be designed to accept 5-mL samples with a water column at least 3 cm deep. The gaseous head space between the water column and the trap must have a total volume of less than 15-mL. The purge gas must pass through the water column as finely divided bubbles with a diameter of less than 3 mm at the origin. The purge gas must be introduced no more than 5 mm from the base of the water column. The sample purger, illustrated in Figure 1, meets these design criteria.

5.2.2 The trap must be at least 25 cm long and have an inside diameter of at least 0.105 inch. The trap must be packed to contain the following minimum lengths of adsorbents: 1.0 cm of methyl silicone coated packing (Section 6.3.2), 15 cm of 2,6-diphenylene oxide polymer (Section 6.3.1), and 8 cm of silica gel, (Section 6.3.3). The minimum specifications for the trap are illustrated in Figure 2.

5.2.3 The desorber should be capable of rapidly heating the trap to 180 °C. The polymer section of the trap should not be heated higher than 180 °C and the remaining sections should not exceed 220 °C. The desorber design, illustrated in Figure 2, meets these criteria.

5.2.4 The purge and trap device may be assembled as a separate unit or be coupled to a gas chromatograph as illustrated in Figures 3 and 4.

5.3 GC/MS system.

5.3.1 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph suitable for on-column injection and all required accessories including syringes, analytical columns, and gases.

5.3.2 Column—6 ft long × 0.1 in ID stainless steel or glass, packed with 1% SP-1000 on Carbopack B (60/80 mesh) or equivalent. This column was used to develop the method performance statements in Section 14. Guidelines for the use of alternate column packings are provided in Section 11.1.

5.3.3 Mass spectrometer—Capable of scanning from 20 to 260 amu every seven seconds or less, utilizing 70 volts (nominal) electron energy in the electron impact ionization mode and producing a mass spectrum which meets all the criteria in Table 2 when 50 ng of 4-bromofluorobenzene (BFB) is injected through the gas chromatograph inlet.

5.3.4 GC/MS interface—Any gas chromatograph to mass spectrometer interface that gives acceptable calibration points at 50 ng or less per injection for each of the parameters of interest and achieves all acceptable performance criteria (see Section 10) may be used. Gas chromatograph to mass spectrometer interfaces constructed of all-glass or glass-lined materials are recommended. Glass can be deactivated by silanizing with dichloro-dimethylsilane.

5.3.5 Data system—A computer system must be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer must have software that allows searching any GC/MS data file for ions of a specified mass and plotting such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundance in any EICP between specified time or scan number limits.

5.4 Syringes—5-mL glass hypodermic with Luerlok tip (two each), if applicable to the purging device.

5.5 Micro syringes—25-mL, 0.006 Inch ID needle.

5.6 Syringe valve—two-way, with Luer ends (three each), if applicable to the purging device.

5.7 Syringe—5-mL, gas-tight with shut-off valve.

5.8 Bottle—15-mL, screw-cap, with Teflon cap liner.

5.9 Balance—Analytical, capable of accurately weighing 0.0001 g.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the MDL of the parameters of interest.

6.1.1 Reagent water may be generated by passing tap water through a carbon filter bed containing about 453 g of activated carbon (Calgon Corp., Filtrasorb-300 or equivalent).

6.1.2 A water purification system (Millipore Super-Q or equivalent) may be used to generate reagent water.

6.1.3 Reagent water may also be prepared by boiling water for 15 minutes. Subsequently, while maintaining the temperature at 90 °C, bubble a contaminant-free inert gas through the water for one hour. While still hot, transfer the water to a narrow-mouth screw-cap bottle and seal with a Teflon-lined septum and cap.

6.2 Sodium thiosulfate—(ACS) Granular.

6.3 Trap materials

6.3.1 2,6-Diphenylene oxide polymer—Tenax (60/80 mesh), chromatographic grade or equivalent.

6.3.2 Methyl silicone packing—3% OV-1 on Chromosorb-W (60/80 mesh) or equivalent.

6.3.3 Silica gel, Davison Chemical, (35/60 mesh), grade-15 or equivalent.

6.4 Methanol—Pesticide quality or equivalent.

6.5 Stock standard solutions—Stock standard solutions may be prepared from pure standard materials or purchased as certified solutions. Prepare stock standard solutions in methanol using assayed liquids or gases as appropriate. Because of the toxicity of some of the organohalides, primary dilutions of these materials should be prepared in a hood. A NIOSH/MESA approved toxic gas respirator should be used when the analyst handles high concentrations of such materials.

6.5.1 Place about 9.8 mL of methanol into a 10-mL ground glass stoppered volumetric flask. Allow the flask to stand, unstoppered, for about 10 minutes or until all alcohol wetted surfaces have dried. Weigh the flask to the nearest 0.1 mg.

6.5.2 Add the assayed reference material as described below:

6.5.2.1 Liquids—Using a 100-µL syringe, immediately add two or more drops of assayed reference material to the flask, then reweigh. The liquid must fall directly into the alcohol without contacting the neck of the flask.

6.5.2.2 Gases—To prepare standards for any of the four halocarbons that boil below 30 °C (bromomethane, chloroethane, chloromethane, and vinyl chloride), fill a 5-mL valved gas-tight syringe with the reference standard to the 5.0-mL mark. Lower the needle to 5 mm above the methanol meniscus. Slowly introduce the reference standard above the surface of the liquid. The heavy gas rapidly dissolves in the methanol.

6.5.3 Reweigh, dilute to volume, stopper, then mix by inverting the flask several times. Calculate the concentration in micrograms per microliter from the net gain in weight. When compound purity is assayed to be 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.5.4 Transfer the stock standard solution into a Teflon-sealed screw-cap bottle. Store, with minimal headspace, at −10° to −20 °C and protect from light.

6.5.5 Prepare fresh standards weekly for the four gases and 2-chloroethylvinyl ether. All other standards must be replaced after one month, or sooner if comparison with check standards indicate a problem.

6.6 Secondary dilution standards—Using stock standard solutions, prepare secondary dilution standards in methanol that contain the compounds of interest, either singly or mixed together. The secondary dilution standards should be prepared at concentrations such that the aqueous calibration standards prepared in Section 7.3.1 or 7.4.1 will bracket the working range of the analytical system. Secondary dilution standards should be stored with minimal headspace and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check standards that can used to determine the accuracy of calibration standards, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.7 Surrogate standard spiking solution—Select a minimum of three surrogate compounds from Table 3. Prepare stock standard solutions for each surrogate standard in methanol as described in Section 6.5. Prepare a surrogate standard spiking solution from these stock standards at a concentration of 150 μg/10 mL in water. Store the spiking solution at 4 °C in Teflon sealed glass containers with a minimum of headspace. The solutions should checked frequently for stability. They should be replaced after six months. The addition of 10 μL of this solution to 5 mL of sample or standard is equivalent to a concentration of 30 μg/L of each surrogate standard. Surrogate standard spiking solutions, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.8 BFB Standard—Prepare a 25 μg/μL solution of BFB in methanol.

7. Calibration

7.1 Assemble a purge and trap device that meets the specifications in Section 5.2. Condition the trap overnight at 180 °C by back flushing with an inert gas flow of at least 20 mL/min. Prior to use, daily condition traps 10 minutes while backflushing at 180 °C.

7.2 Connect the purge and trap device to a gas chromatograph. The gas chromatograph must be operated using temperature and flow rate parameters equivalent to those in Table 1. Calibrate the purge and trap-GC/MS system using either the external standard technique (Section 7.3) or the internal standard technique (Section 7.4).

7.3 External standard calibration procedure:

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter by carefully adding 20.0 μL of one or more secondary dilution standards to 50, 250, or 500 mL of reagent water. A 25-μL syringe with a 0.006 inch ID needle should be used for this operation. One of the external standards should be at a concentration near, but above, the MDL (See Table 1) and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC/MS system. Aqueous standards may be stored up to 24 hours, if held in sealed vials with zero headspace as described in Section 9.2. If not so stored, they must be discarded after one hour.

7.3.2 Analyze each calibration standard according to Section 11, and tabulate the area response of the primary characteristic ion (See Table 4) against the concentration in the standard. The results can be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to concentration (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin can be assumed and the average ratio or calibration factor can be used in place of a calibration curve.

7.3.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ± 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that parameter.

7.4 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples. Due to their generally unique retention times, bromochloromethane, 2-bromo-1-chloropropane, and 1,4-dichlorobutane have been used successfully as internal standards.

7.4.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest as described in Section 7.3.1.

7.4.2 Prepare a spiking solution containing each of the internal standards using the procedures described in Sections 6.5 and 6.6. It is recommended that the secondary dilution standard be prepared at a concentration of 15 μg/mL of each internal standard compound. The addition of 10 μL of this standard to 5.0 mL of sample or calibration standard would be equivalent to 30 μg/L.

7.4.3 Analyze each calibration standard, according to Section 11, adding 10 μL of internal standard spiking solution directly to the syringe (Section 11.4). Tabulate the area response of the characteristic ions against concentration for each compound and internal standard and calculate response factors (RF) for each compound using equation 1.

Eq. 1 $RF = (A_s C_{is})/(A_{is} C_s)$

where:

A_s = Area of the characteristic ion for the parameter to be measured.

A_{is} = Area of the characteristic ion for the internal standard.

C_{is} = Concentration of the internal standard.

C_s = Concentration of the parameter to be measured.

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve or response ratios, A_s/A_{is}, vs. RF.

7.4.4 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ± 10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared for that compound.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted to certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike all samples with surrogate standards to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each parameter to be measured. Using stock standards, prepare a quality control check sample concentrate in methanol 500 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a syringe, add 10 µL of the check sample concentrate and 10 µL of the surrogate standard dosing solution (Section 6.7) to each of a minimum of four 5-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 11.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for all parameters and surrogate standards. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 5, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > p$ or $|X - R| > p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the results of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria for each of the surrogate standards.

8.3.1 Calculate upper and lower control limits for method performance for each surrogate standard, using the values for R and s calculated in Section 8.2.3:

Upper Control Limit (UCL) = $R + 3s$
Lower Control Limit (LCL) = $R - 3s$

The UCL and LCL can be used to construct control charts(8) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 For each surrogate standard, the laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly(8).

8.4 The laboratory is required to spike all of their samples with the surrogate standard spiking solution to monitor spike recoveries. If the recovery for any surrogate standard does not fall within the control limits for method performance, the results reported for that sample must be qualified as described in Section 13.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Each day, the analyst must demonstrate, through the analysis of reagent water, that interferences from the analytical system are under control.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 All samples must be iced or refrigerated from the time of collection until extraction. If the sample contains residual chlorine, add sodium thiosulfate preservative (10 mg/40 mL is sufficient for up to 5 ppm Cl_2) to the empty sample bottles just prior to shipping to the sampling site. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used for measurement of residual chlorine(9). Field test kits are available for this purpose.

9.2 Grab samples must be collected in glass containers having a total volume of at least 25 mL. Fill the sample bottle just to overflowing in such a manner that no air bubbles pass through the sample as the bottle is being filled. Seal the bottle so that no air bubbles are entrapped in it. If preservative has been added, shake vigorously for one minute. Maintain the hermetic seal on the sample bottle until time of analysis.

9.3 Experimental evidence indicates that some aromatic compounds, notably benzene, toluene, and ethyl benzene are susceptible to rapid biological degradation under certain environmental conditions(3). Refrigeration along may not be adequate to preserve these compounds in wastewaters for more than seven days. For this reason, a separate sample should be collected, acidified, and analyzed when these aromatics are to be determined. Collect about 500 mL of sample in a clean container. Adjust the pH of the sample to about 2 by adding HCl (1 + 1) while stirring. Check pH with narrow range (1.4 to 2.8) pH paper. Fill a sample container as described in Section 9.2. If chlorine residual is present, add sodium thiosulfate to another sample container and fill as in Section 9.2 and mix thoroughly.

9.4 All samples must be analyze within 14 days of collection.

10. Daily GC/MS Performance Tests

10.1 At the beginning of each day that analyses are to be performed, the

GC/MS system must be checked to see if acceptable performance criteria are achieved for BFB(10). The performance test must be passed before any samples, blanks, or standards are analyzed, unless the instrument has met the DFTPP test described in method 625 earlier in the day(11).

10.2 These performance tests require the following instrumental parameters.

Electron Energy: 70 Volts (nominal)
Mass Range: 20 to 260
Scan Time: to give at least 5 scans per peak but not to exceed 7 seconds per scan.

10.3 At the beginning of each day, inject 2 µL of BFB solution directly on column. Alternately, add 2 µL of BFB solution to 5.0 mL of reagent water or standard solution and analyze according to Section 11. Obtain a background corrected mass spectrum of BFB and check that all the key ion criteria in Table 2 are achieved. If all the criteria are not achieved, the analyst must retune the mass spectrometer and repeat the test until all criteria are achieved.

11. Sample Extraction and Gas Chromatography

11.1 Table 1 summarizes the recommended operating conditions for the gas chromatograph. This table includes retention times and method detection limits that were achieved under these conditions. An example of the parameter separations achieved by Column 1 is shown in Figure 5. Other packed columns or chromatographic conditions may be used if the requirements of Section 8.2 are met.

11.2 After achieving the key ion abundance criteria in Section 10, calibrate the system daily as described in Section 7.

11.3 Adjust the purge gas (helium) flow rate to 40 ± 3 mL/min. Attach the trap inlet to the purging device, and set the device to purge. Open the syringe valve located on the purging device sample introduction needle.

11.4 Remove the plunger from a 5-mL syringe and attach a closed syringe valve. Open the sample or standard bottle which has been allowed to come to ambient temperature, and carefully pour the sample into the syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. Since this process of taking an aliquot destroys the validity of the sample for future analysis, the analyst should fill a second syringe at this time to protect against possible loss of data. Add 10.0 µL of the surrogate spiking solution (Section 6.7) and, if applicable, 10.0 µL of the internal standard spiking solution (Section 7.4.2) through the valve bore, then close the valve. The surrogate and internal standards may be mixed and added as a single spiking solution.

11.5 Attach the syringe-syringe valve assembly to the syringe valve on the purging device. Open the syringe valves and inject the sample into the purging chamber.

11.6 Close both valves and purge the sample for 11.0 ± 0.1 minutes at ambient temperature.

11.7 At the conclusion of the purge time, attach the trap to the chromatograph, adjust the device to the desorb mode, and begin the gas chromatographic temperature program. Concurrently, introduce the trapped materials to the gas chromatographic column by rapidly heating the trap to 180 °C while backflushing the trap with an inert gas between 20 and 60 mL/min for four minutes. If this rapid heating requirement cannot be met, the gas chromatographic column must be used as a secondary trap by cooling it to 30 °C (or subambient, if problems persist) instead of the recommended initial temperature of 45 °C.

11.8 While the trap is being desorbed into the gas chromatograph, empty the purging chamber using the sample introduction syringe. Wash the chamber with two 5-mL flushes of reagent water.

11.9 After desorbing the sample for four minutes, recondition the trap by returning the purge and trap device to the purge mode. Wait 15 seconds then close the syringe valve on the purging device to begin gas flow through the trap. The trap temperature should be maintained at 180 °C. Trap temperatures up to 230 °C may be employed, however, the higher temperature will shorten the useful life of the trap. After approximately seven minutes turn off the trap heater and open the syringe valve to stop the gas flow through the trap. When cool, the trap is ready for the next sample.

11.10 If the response for any ion exceeds the working range of the system, dilute the sample aliquot in the second syringe with reagent water and reanalyze.

12. Qualitative Identification

12.1 Obtain EICPs for the primary ion (Table 4) and at least two secondary ions for each parameter of interest. The following criteria must be met to make a qualititative identification.

12.1.1 The characteristic ions of each parameter of interest must maximize in the same or within one scan of each other.

12.1.2 The retention time must fall within ± 30 seconds of the retention time of the authentic compound.

12.1.3 The relative peak heights of the three characteristic ions in the EICPs must fall within ± 20% of the relative intensities of these ions in a reference mass spectrum. The reference mass spectrum can be obtained from a standard analyzed in the GC/MS system or from a reference library.

12.2 Structural isomers that have very similar mass spectra and less than 30 seconds difference in retention time, can be explicitly identified only if the resolution between authentic isomers in a standard mix is acceptable. Acceptable resolution is achieved if the baseline to valley height between the isomers is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

13. Calculations

13.1 When a parameter has been identified, the quantitation of that parameter should be based on the integrated abundance from the EICP of the first listed characteristic ion given in Table 4. If the sample produces an interference for the primary ion, use a secondary characteristic ion to quantitate. Quantitation may be performed using the external or internal standard techniques.

13.1.1 If the external standard calibration procedure is used, calculate the concentration of the parameter being measured from the area of the characteristic ion using the calibration curve or calibration factor in Section 7.3.2.

13.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.4.3 and equation 2.

Eq. 2.

$$\text{Concentration } \mu g/L = (A_s C_{is})/(A_{is})(RF)$$

where:

A_s = Area of the characteristic ion for the parameter or surrogate standard to be measured.
A_{is} = Area of the characteristic ion for the internal standard.
C_{is} = Concentration of the internal standard.

13.2 Report results in micrograms per liter. The results for cis- and trans-1,3 dichloropropene should be reported as total 1,3-dichloropropene (STORET No. 34561, CAS No. 542-75-6). When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

13.3 If any of the surrogate standard recoveries fall outside the control limits which were established as directed in Section 8.4, data for all parameters determined by this method in that sample must be labeled as suspect.

14. Method Performance

14.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero(1). The MDL concentrations listed in Table 1 were obtained using reagent water(12). Similar results were achieved using representative wastewaters.

14.2 The average recoveries and the average standard deviations of the percent recoveries, presented in Table 5, were the result of a study of the accuracy and precision of this method by several laboratories. The values listed represent the results from 2 to 4 laboratories(13).

14.3 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

References

1. See Appendix A.
2. Bellar, T.A., and J.J. Lichtenberg, *Journal American Water Works Association, 66,* p. 739, (1974).
3. Bellar, T.A., and J.J. Lichtenberg, "Semi-Automated Headspace Analysis of Drinking Waters and Industrial Waters for Purgeable Volatile Organic Compounds," Measurement of Organic Pollutants in Water and Wastewater, C.E. Van Hall, editor, American Society for Testing and Materials, Philadelphia, PA. Special Technical Publication 686, 1978.
4. "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants." U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, March 1977, Revised April 1977. Effluent Guidelines Division, Washington, DC 10460.
5. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.
6. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).
7. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.
8. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
9. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600/4-79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.
10. Budde, W.L. and Eichelberger, J.W., "Performance Tests for the Evaluation of Computerized Gas Chromatography/Mass Spectrometry Equipment and Laboratories," EPA-600/4-80-025, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268, p. 16, April 1980.
11. Eichelberg, J.W. Harris, L.E., and Budde, W.L., "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography—Mass Spectrometry Systems,", *Analytical Chemistry, 47,* 995-1000 (1979).
12. "Method Detection Limit for Methods 624 and 625," Olynyk, P., Budde, W.L. Eichelberger, J.W., unpublished report, October 1980.
13. Kleopfer, R.D., "POTW Toxic Study, Analytical Quality Assurance Final Report," U.S. Environmental Protection Agency, Region VII, Kansas City, Kansas 66115, 1981.

Table 1. Chromatographic Conditions and Method Detection Limits

Parameter	*Retention Time (min.) Column 1*	*Method Detection Limit (µg/L)*
Chloromethane	*2.3*	*nd*
Bromomethane	*3.1*	*nd*
Vinyl chloride	*3.8*	*nd*
Chloroethane	*4.6*	*nd*
Methylene chloride	*6.4*	*2.8*
Trichlorofluoromethane	*8.3*	*nd*
1,1-Dichloroethene	*9.0*	*2.8*
1,1-Dichloroethane	*10.1*	*4.7*
trans-1,2-Dichloroethene	*10.8*	*1.6*
Chloroform	*11.4*	*1.6*
1,2-Dichloroethane	*12.1*	*2.8*
1,1,1-Trichloroethane	*13.4*	*3.8*
Carbon tetrachloride	*13.7*	*2.8*
Bromodichloromethane	*14.3*	*2.2*
1,2-Dichloropropane	*15.7*	*6.0*
trans-1,3-Dichloropropene	*15.9*	*5.0*
Trichloroethene	*16.5*	*1.9*
Benzene	*17.0*	*4.4*
Dibromochloromethane	*17.1*	*3.1*
1,1,2-Trichloroethane	*17.2*	*5.0*
cis-1,3-Dichloropropene	*17.2*	*nd*
2-Chloroethylvinyl ether	*18.6*	*nd*
Bromoform	*19.8*	*4.7*
1,1,2,2-Tetrachloroethane	*22.1*	*6.9*
Tetrachloroethene	*22.2*	*4.1*
Toluene	*23.5*	*6.0*
Chlorobenzene	*24.6*	*6.0*
Ethyl benzene	*26.4*	*7.2*
1,3-Dichlorobenzene	*33.9*	*nd*
1,2-Dichlorobenzene	*35.0*	*nd*
1,4-Dichlorobenzene	*35.4*	*nd*

nd = not determined

Column conditions: Carbopak B (60/80 mesh) coated with 1% SP-1000 packed in a 6 ft by 2 mm ID glass column with helium carrier gas at a flow rate of 30 mL/min. Column temperature is isothermal at 45°C for 3 min, then programmed at 8°C per minute to 220°C and held for 15 min.

Table 2. BFB Key Ion Abundance Criteria

Mass	*Ion Abundance Criteria*
50	*15 to 40% of mass 95*
75	*30 to 60% of mass 95*
95	*Base Peak, 100% Relative Abundance*
96	*5 to 9% of mass 95*
173	*<2% of mass 174*
174	*>50% of mass 95*
175	*5 to 9% of mass 174*
176	*>95% but < 101% of mass 174*
177	*5 to 9% of mass 176*

Table 3. *Suggested Surrogate and Internal Standards*

Compound	*Retention Time (min.)*[a]	*Primary Ion*	*Secondary Ions*
Surrogate Standards			
Benzene d-6	*17.0*	*84*	—
4-Bromofluorobenzene	*28.3*	*95*	*174, 176*
1,2-Dichloroethane d-4	*12.1*	*102*	—
1,4-Difluorobenzene	*19.6*	*114*	*63, 88*
Ethylbenzene d-5	*26.4*	*111*	—
Ethylbenzene d-10	*26.4*	*98*	—
Fluorobenzene	*18.4*	*96*	*70*
Pentafluorobenzene	*23.5*	*168*	—
Internal Standards			
Bromochloromethane	*9.3*	*128*	*49, 130, 51*
2-Bromo-1-chloropropane	*19.2*	*77*	*79, 156*
1,4-Dichlorobutane	*25.8*	*55*	*90, 92*

[a] *For chromatographic conditions, see Table 1.*

Table 4. *Characteristic Ions for Purgeable Organics*

Parameter	*Primary Ion*	*Secondary Ions*
Chloromethane	*50*	*52*
Bromomethane	*94*	*96*
Vinyl chloride	*62*	*64*
Chloroethane	*64*	*66*
Methylene chloride	*84*	*49, 51, 86*
Trichlorofluoromethane	*101*	*103*
1,1-Dichloroethene	*96*	*61, 98*
1,1-Dichloroethane	*63*	*65, 83, 85, 98, 100*
trans-1,2-Dichloroethene	*96*	*61, 98*
Chloroform	*83*	*85*
1,2-Dichloroethane	*98*	*62, 64, 100*
1,1,1-Trichloroethane	*97*	*99, 117, 119*
Carbon tetrachloride	*117*	*119, 121*
Bromodichloromethane	*127*	*83, 85, 129*
1,2-Dichloropropane	*112*	*63, 65, 114*
trans-1,3-Dichloropropene	*75*	*77*
Trichloroethene	*130*	*95, 97, 132*
Benzene	*78*	
Dibromochloromethane	*127*	*129, 208, 206*
1,1,2-Trichloroethane	*97*	*83, 85, 99, 132, 134*
cis-1,3-Dichrloropropene	*75*	*77*
2-Chloroethylvinyl ether	*106*	*63, 65*
Bromoform	*173*	*171, 175, 250, 252, 254, 256*
1,1,2,2-Tetrachloroethane	*168*	*83, 85, 131, 133, 166*
Tetrachloroethene	*164*	*129, 131, 166*
Toluene	*92*	*91*
Chlorobenzene	*112*	*114*
Ethyl benzene	*106*	*91*
1,3-Dichlorobenzene	*146*	*148, 113*
1,2-Dichlorobenzene	*146*	*148, 113*
1,4-Dichlorobenzene	*146*	*148, 113*

Table 5. *Accuracy and Precision for Purgeable Organics*

Parameter	Reagent Water Average Percent Recovery	Reagent Water Standard Deviation (%)	Wastewater Average Percent Recovery	Wastewater Standard Deviation (%)
Benzene	99	9	98	10
Bromodichloromethane	102	12	103	10
Bromoform	104	14	105	16
Bromomethane	100	20	88	23
Carbon tetrachloride	102	16	104	15
Chlorobenzene	100	7	102	9
Chloroethane	97	22	103	31
2-Chloroethylvinyl ether	101	13	95	17
Chloroform	101	10	101	12
Chloromethane	99	19	99	24
Dibromochloromethane	103	11	104	14
1,1-Dichloroethane	101	10	104	15
1,2-Dichloroethane	100	8	102	10
1,1-Dichloroethene	102	17	99	15
trans-1,2-Dichloroethene	99	12	101	10
1,2-Dichloropropane	102	8	103	12
cis-1,3-Dichloropropene	105	15	102	19
trans-1,3-Dichloropropene	104	11	100	18
Ethyl benzene	100	8	103	10
Methylene chloride	96	16	89	28
1,1,2,2-Tetrachloroethane	102	9	104	14
Tetrachloroethene	101	9	100	11
Toluene	101	9	98	14
1,1,1-Trichloroethane	101	11	102	16
1,1,2-Trichloroethane	101	10	104	15
Trichloroethene	101	9	100	12
Trichlorofluoromethane	103	11	107	19
Vinyl chloride	100	13	98	25

Samples were spiked between 10 and 1000 μg/L.

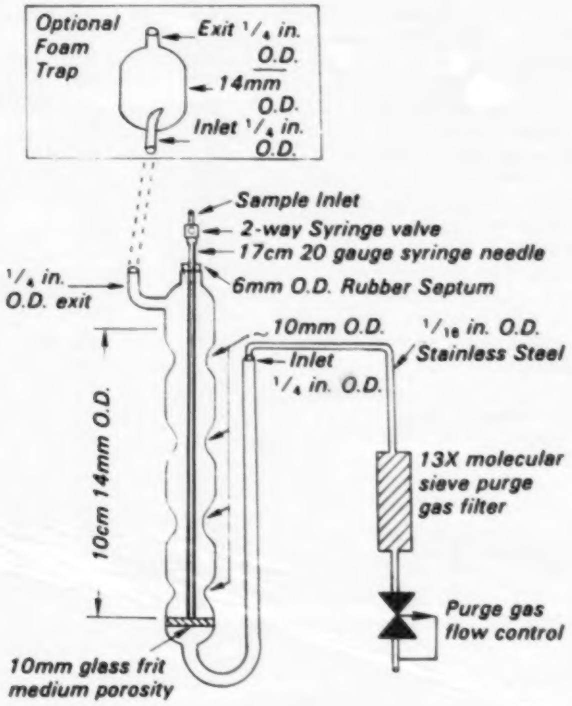

Optional Foam Trap
Exit 1/4 in. O.D.
14mm O.D.
Inlet 1/4 in. O.D.
Sample Inlet
2-way Syringe valve
17cm 20 gauge syringe needle
6mm O.D. Rubber Septum
1/4 in. O.D. exit
10mm O.D.
1/16 in. O.D. Stainless Steel
Inlet 1/4 in. O.D.
10cm 14mm O.D.
13X molecular sieve purge gas filter
Purge gas flow control
10mm glass frit medium porosity

Figure 1. *Purging device*

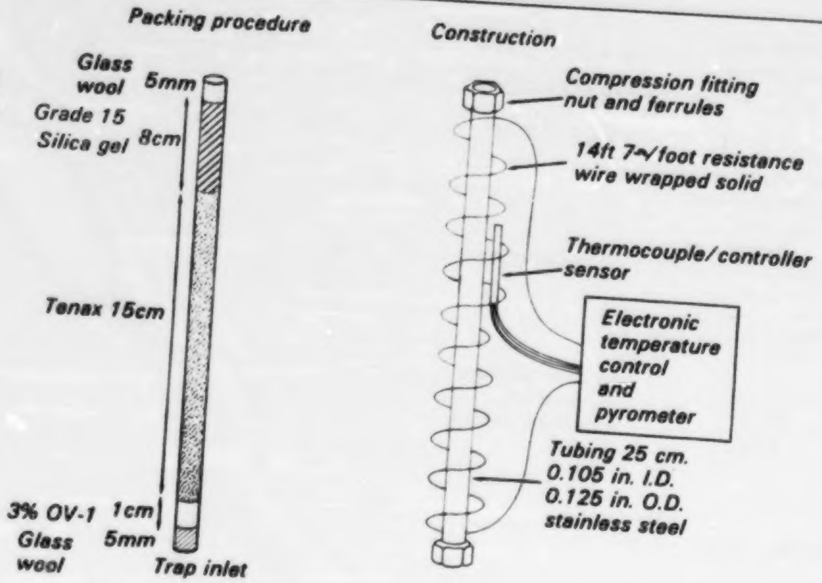

Packing procedure
Construction
Glass wool 5mm
Grade 15 Silica gel 8cm
Tenax 15cm
3% OV-1 1cm
Glass wool 5mm
Trap inlet
Compression fitting nut and ferrules
14ft 7Ω/foot resistance wire wrapped solid
Thermocouple/controller sensor
Electronic temperature control and pyrometer
Tubing 25 cm.
0.105 in. I.D.
0.125 in. O.D.
stainless steel

Figure 2. *Trap packings and construction to include desorb capability*

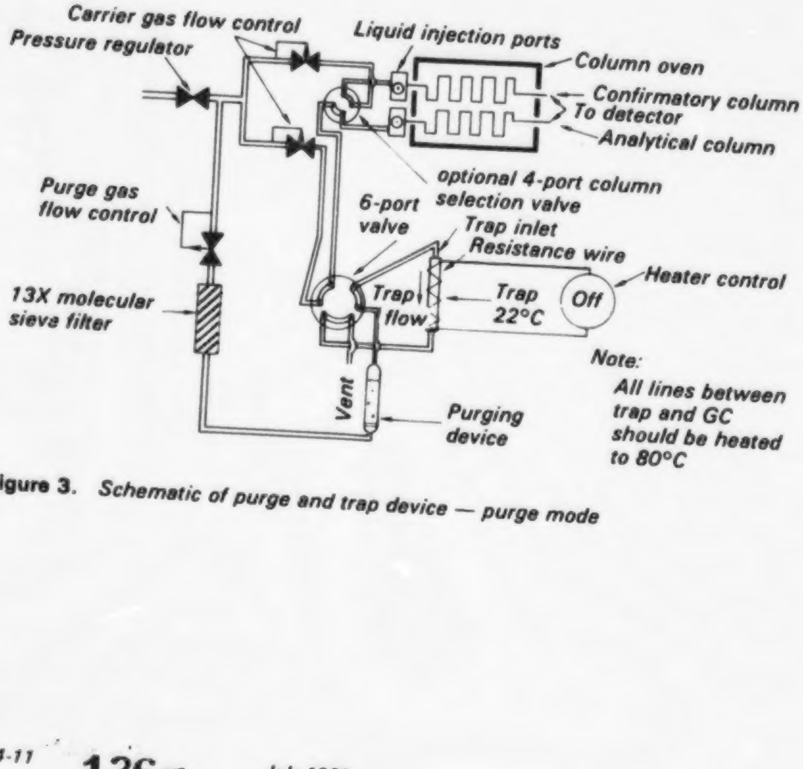

Carrier gas flow control
Pressure regulator
Liquid injection ports
Column oven
Confirmatory column
To detector
Analytical column
optional 4-port column selection valve
Purge gas flow control
6-port valve
Trap inlet
Resistance wire
Heater control
Off
13X molecular sieve filter
Trap flow
Trap 22°C
Vent
Purging device
Note:
All lines between trap and GC should be heated to 80°C

Figure 3. *Schematic of purge and trap device — purge mode*

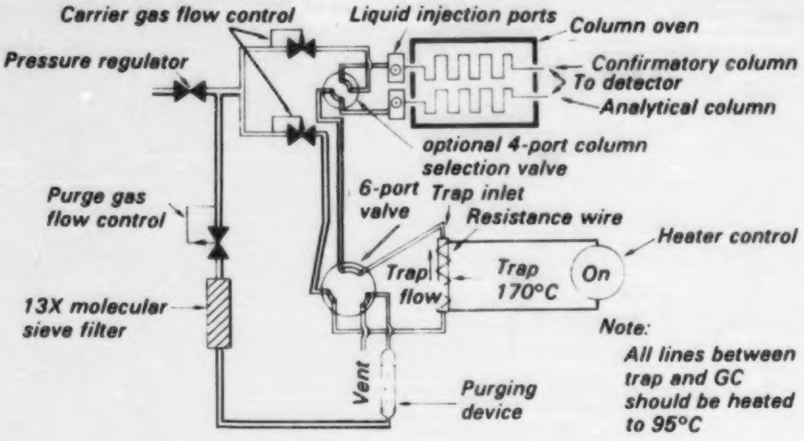
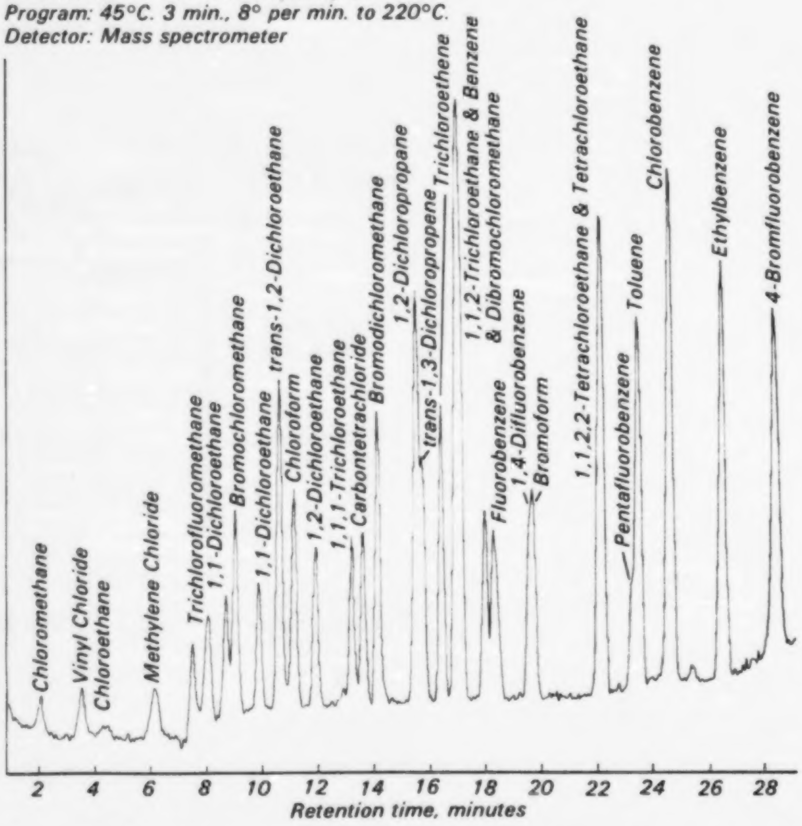

Carrier gas flow control
Liquid injection ports
Column oven
Pressure regulator
Confirmatory column
To detector
Analytical column
optional 4-port column selection valve
Purge gas flow control
6-port valve
Trap inlet
Resistance wire
Heater control
Trap flow
Trap 170°C
On
13X molecular sieve filter
Vent
Purging device
Note:
All lines between trap and GC should be heated to 95°C

Figure 4. *Schematic of purge and trap device — desorb mode*



Column: 1% SP-1000 on Supelcoport
Program: 45°C. 3 min., 8° per min. to 220°C.
Detector: Mass spectrometer

Figure 5. *Gas chromatogram of volatile organics by purge and trap.*

United States
Environmental Protection
Agency

Environmental Monitoring and
Support Laboratory
Cincinnati OH 45268

Research and Development

EPA

Test Method

Base/Neutrals and Acids — Method 625

1. Scope and Application

1.1 This method covers the determination of a number of organic compounds that are partitioned into an organic solvent and are amenable to gas chromatography. The parameters listed in Tables 1 and 2 may be qualitatively and quantitatively determined using this method.

1.2 The method may be extended to include the parameters listed in Table 3. Benzidine can be subject to oxidative losses during solvent concentration. α-BHC, γ-BHC, endosulfan I and II, and endrin are subject to decomposition under the alkaline conditions of the extraction step. Hexachlorocyclopentadiene is subject to thermal decomposition in the inlet of the gas chromatograph, chemical reaction in acetone solution and photochemical decomposition. N-nitrosodimethylamine is difficult to separate from the solvent under the chromatographic conditions described. N-nitrosodiphenylamine decomposes in the gas chromatographic inlet and cannot be separated from diphenylamine. The preferred method for each of these parameters is listed in Table 3.

1.3 This is a gas chromatography/mass spectrometry (GC/MS) method applicable to the determination of the compounds listed in Tables 1, 2, and 3 in municipal and industrial discharges as provided under 40 CFR 136.1. Until the U.S. Environmental Protection Agency establishes performance criteria based upon the results of interlaboratory testing, any alternative GC/MS method which meets the performance criteria described in Section 8.2 will be permitted. Performance must be verified for such modification by analyzing wastewater as described in Section 8.2.2. In addition, the laboratory must successfully participate in the applicable performance evaluation studies.

1.4 The method detection limit (MDL, defined in Section 16)(1) for each parameter is listed in Tables 4 and 5. The MDL for a specific wastewater differ from those listed, depending upon the nature of interferences in the sample matrix.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the operation of gas chromatograph/mass spectrometers and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedure described in Section 8.2.

2. Summary of Method

2.1 A measured volume of sample, approximately one-liter, is serially extracted with methylene chloride at a pH greater than 11 and again at pH less than 2 using a separatory funnel or a continuous extractor. The methylene chloride extract is dried and concentrated to a volume of 1 mL. Chromatographic conditions are described which permit the separation and measurement of the parameters in the extract. Qualitative identification is performed using the retention time and the relative abundance of three characteristic ions. Quantitative analysis is performed using either external or internal standard techniques with a single characteristic ion.

3. Interferences

3.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the total ion current profiles. All of these materials must be routinely demonstrated to be free from interferences under the conditions of the analysis by running laboratory reagent blanks as described in Section 8.5.

3.1.1 Glassware must be scrupulously cleaned[3]. Clean all glassware as soon as possible after use by rinsing with the last solvent used in it. This should be followed by detergent washing with hot water, and rinses with tap water and reagent water. It should then be drained dry, and heated in a muffle furnace at 400 °C for 15 to 30 minutes. Some thermally stable materials, such as PCBs, may not be eliminated by this treatment. Solvent rinses with acetone and pesticide quality hexane may be substituted for the muffle furnace heating. Volumetric ware should not be heated in a muffle furnace. After drying and cooling, glassware should be sealed and stored in a clean environment to prevent any accumulation of dust or other contaminants. Store it inverted or capped with aluminum foil.

3.1.2 The use of high purity reagents and solvents helps to minimize interference problems. Purification of solvents by distillation in all-glass systems may be required.

3.2 Matrix interferences may be caused by contaminants that are coextracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled.

3.3 The base-neutral extraction may cause significantly reduced recovery of phenol, 2-methylphenol, and 2,4-dimethylphenol. The analyst must recognize that results obtained under these conditions are minimum concentrations.

3.4 The packed gas chromatographic columns recommended for the basic fraction may not exhibit sufficient resolution for certain isomeric pairs. These include anthracene and phenanthrene; chrysene and benzo(a)anthracene; and benzo(b)fluoranthene and benzo(k)fluoranthene. The gas chromatograph retention time and mass spectra are not sufficiently different to make an unambiguous distinction between these compounds. Alternative techniques should be used to identify and quantify these specific compounds. See method 610.

3.5 In samples that contain an inordinate number of interferences, the use of chemical ionization (CI) mass spectrometry may make identification easier. Tables 6 and 7 give characteristic CI ions for most of the compounds covered by this method. The use of CI mass spectrometry to support electron ionization (EI) mass spectrometry is encouraged but not required.

4. Safety

4.1 The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available and have been identified for the information of the analyst[4-6].

4.2 The following parameters covered by this method have been tentatively classified as known or suspected, human or mammalian carcinogens; benzo(a)anthracene, benzidine, 3,3′-dichlorobenzidine, benzo(a)pyrene, α-BHC, β-BHC, δ-BHC, γ-BHC, dibenzo(a,h) anthracene, N-nitrosodimethylamine, 4,4′-DDT and polychlorinated biphenyls.

5. Apparatus and Materials

5.1 Sampling equipment, for discrete or composite sampling.

5.1.1 Grab sample bottle—Amber glass, one-liter or one-quart volume, fitted with screw caps lined with Teflon. Foil may be substituted for Teflon if the sample is not corrosive. If amber bottles are not available, protect samples from light. The container must be washed, rinsed with acetone or methylene chloride, and dried before use to minimize contamination.

5.1.2 Automatic sampler (optional)—Must incorporate glass sample containers for the collection of a minimum of 250 mL. Sample containers must be kept refrigerated at 4 °C and protected from light during compositing. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used. Before use, however, the compressible tubing should be thoroughly rinsed with methanol, followed by repeated rinsings with distilled water to minimize the potential for contamination of the sample. An integrating flow meter is required to collect flow proportional composites.

5.2 Glassware (All specifications are suggested. Catalog numbers are included for illustration only).

5.2.1 Separatory funnel—2000-mL, with Teflon stopcock.

5.2.2 Drying column—19 mm ID chromatographic column with coarse frit.

5.2.3 Concentrator tube, Kuderna-Danish—10-mL, graduated (Kontes K-570050-1025 or equivalent). Calibration must be checked at the volumes employed in the test. Ground glass stopper is used to prevent evaporation of extracts.

5.2.4 Evaporative flask, Kuderna-Danish—500-mL (Kontes K-570001-0500 or equivalent). Attach to concentrator tube with springs.

5.2.5 Snyder column, Kuderna-Danish—Three-ball macro (Kontes K-503000-0121 or equivalent).

5.2.6 Snyder column, Kuderna-Danish—Two-ball micro (Kontes K-569001-0219 or equivalent).

5.2.7 Vials—Amber glass, 10- to 15-mL capacity, with Teflon-lined screw cap.

5.2.8 Continuous liquid-liquid extractors—Equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication. (Hershberg-Wolf Extractor-Ace Glass Company, Vineland, N.J. P/N 6841-10 or equivalent.)

5.3 Boiling chips—approximately 10/40 mesh. Heat to 400 °C for 30 minutes or Soxhlet extract with methylene chloride.

5.4 Water bath—Heated, with concentric ring cover, capable of temperature control (± 2 °C). The bath should be used in a hood.

5.5 Balance—Analytical, capable of accurately weighing 0.0001 g.

5.6 GC/MS system.

5.6.1 Gas chromatograph—An analytical system complete with a temperature programmable gas chromatograph and all required accessories including syringes, analytical columns, and gases. The injection port must be designed for on-column injection when using packed columns and for splitless injection when using capillary columns.

5.6.2 Column for Base Neutrals—1.8 m long × 2 mm ID glass, packed with Supelcoport (100/120 mesh) coated with 3% SP-2250 or equivalent. This column was used to develop the accuracy and precision statements in Table 6 and the MDL data in Table 4. Guidelines for the use of alternate column packings are provided in Section 13.1.

5.6.3 Column for Acids—1.8 m long × 2 mm ID glass, packed with Supelcoport (100/120 mesh) coated with 1% SP-1240 DA or equivalent. This column was used to develop the accuracy and precision statements in Table 7, and the MDL data in Table 5. Guidelines for the use of alternate column packings are given in Section 13.1.

5.6.4 Mass Spectrometer—Capable of scanning from 35 to 450 amu every seven seconds or less utilizing a 70 volt (nominal) electron energy in the electron impact ionization mode and producing a mass spectrum which meets all the criteria in Table 9 when 50 ng of decafluorotriphenyl phosphine (DFTPP; bis(perfluorophenyl) phenyl phosphine) is injected through the gas chromatographic inlet. Any gas chromatograph to mass spectrometer interface that gives acceptable calibration points at 50 ng per injection for each compound of interest in Tables 1 through 3 and achieves all acceptable performance criteria (Section 12) may be used. Gas chromatograph to mass spectrometer interfaces constructed of all glass or glass lined materials are recommended. Glass can be deactivated by silanizing with dichlorodimethylsilane.

5.6.5 A computer system must be interfaced to the mass spectrometer that allows the continuous acquisition and storage on machine readable media of all mass spectra obtained throughout the duration of the chromatographic program. The computer must have software that allows searching any GC/MS data file for ions of a specific mass and plotting such ion abundances versus time or scan number. This type of plot is defined as an Extracted Ion Current Profile (EICP). Software must also be available that allows integrating the abundance in any EICP between specified time or scan number limits.

6. Reagents

6.1 Reagent water—Reagent water is defined as a water in which an interferent is not observed at the method detection limit of each parameter of interest.

6.2 Sodium hydroxide solution (10 N)—Dissolve 40g NaOH in reagent water and dilute to 100 mL.

6.3 Sodium thiosulfate—(ACS) Granular.

6.4 Sulfuric acid solution (1 + 1)—Slowly add 50 mL of H_2SO_4 (sp. gr. 1.84) to 50 mL of reagent water.

6.5 Acetone, methanol, methylene chloride—Pesticide quality or equivalent.

6.6 Sodium sulfate—(ACS) Granular, anhydrous. Purify by heating at 400 °C for four hours in a shallow tray.

6.7 Stock standard solutions (1.00 µg/µL)—Stock standard solutions can be prepared from pure standard materials or purchased as certified solutions.

6.7.1 Prepare stock standard solutions by accurately weighing about 0.0100 g of pure material. Dissolve the material in pesticide quality acetone or other suitable solvent and dilute to volume in a 10-mL volumetric flask. Larger volumes may be used at the convenience of the analyst. If compound purity is assayed at 96% or greater, the weight may be used without correction to calculate the concentration of the stock standard. Commercially prepared stock standards may be used at any concentration if they are certified by the manufacturer or by an independent source.

6.7.2 Transfer the stock standard solutions into Teflon-sealed screw-cap bottles. Store in at 4 °C and protect from light. Stock standard solutions should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them. Quality control check samples, that can be used to determine the accuracy of calibration standards will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.7.3 Stock standard solutions must be replaced after six months or sooner if comparison with quality control check samples indicate a problem.

6.8 Surrogate standard spiking solutions—select a minimum of three surrogate compounds from Table 8. Prepare a surrogate standard spiking solution at a concentration of 100 µg/1.00 ML in acetone. Addition of 1.00 mL of this solution to 1000-mL of sample is equivalent to a concentration of 100 µg/L of each surrogate standard. Store the spiking solutions at 4 °C in Teflon-sealed containers. The solutions should be checked frequently for stability. These solutions must be replaced after six months, or sooner if comparison with quality control check samples indicate a problem. Surrogate standard spiking solutions, appropriate for use with this method will be available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

6.9 DFTPP standard—Prepare a 25 ng/µL solution of DFTPP in acetone.

7. Calibration

7.1 Establish gas chromatographic operating parameters equivalent to those indicated in Tables 4 or 5. The GC/MS system can be calibrated using the external standard technique (Section 7.2) or the internal standard technique (Section 7.3).

7.2 External standard calibration procedure:

7.2.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with acetone. One of the external standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC/MS system.

7.2.2 Analyze 2 to 5 µL of each calibration standard and tabulate the area responses of the primary characteristic ion of each standard (Tables 4 and 5) against the mass injected. The results may be used to prepare a calibration curve for each compound. Alternatively, if the ratio of response to amount injected (calibration factor) is a constant over the working range (<10% relative standard deviation, RSD), linearity through the origin may be assumed and the average ratio or calibration factor may be used in place of a calibration curve.

7.2.3 The working calibration curve or calibration factor must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve or calibration factor must be prepared for that compound.

7.3 Internal standard calibration procedure. To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Table 8 lists some recommended internal standards. Phenanthrene-d_{10} has been used for this purpose. Use the base peak ion as the primary ion for quantification of the standards. If interferences are noted, use the next two most intense ions as the secondary ions.

7.3.1 Prepare calibration standards at a minimum of three concentration levels for each parameter of interest by adding appropriate volumes of one or more stock standards to a volumetric flask. To each calibration standard or standard mixture, add a known constant amount of one or more internal standards, and dilute to volume with acetone. One of the calibration standards should be at a concentration near, but above, the MDL and the other concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC/MS system.

7.3.2 Analyze 2 to 5 µL of each calibration standard and tabulate the area of the primary characteristic ion (Tables 4 and 5) against concentration for each compound and internal standard, and calculate response factors (RF) for each compound using equation 1.

$$\text{Eq. 1}\quad RF = (A_sC_{is})/(A_{is}C_s)$$

where:

A_s = Area of the characteristic ion for the parameter to be measured.
A_{is} = Area of the characteristic ion for the internal standard.
C_{is} = Concentration of the internal standard, (µg/L).
C_s = Concentration of the parameter to be measured, (µg/L).

If the RF value over the working range is a constant (<10% RSD), the RF can be assumed to be invariant and the average RF can be used for calculations. Alternatively, the results can be used to plot a calibration curve of response ratios, A_s/A_{is}, vs. RF.

7.3.3 The working calibration curve or RF must be verified on each working day by the measurement of one or more calibration standards. If the response for any parameter varies from the predicted response by more than ±10%, the test must be repeated using a fresh calibration standard. Alternatively, a new calibration curve must be prepared.

8. Quality Control

8.1 Each laboratory that uses this method is required to operate a formal quality control program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the analysis of spiked samples as a continuing check on performance. The laboratory is required to maintain performance records to define the quality of data that is generated. Ongoing performance checks must be compared with established performance criteria to determine if the results of analyses are within accuracy and precision limits expected of the method.

8.1.1 Before performing any analyses, the analyst must demonstrate the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 8.2.

8.1.2 In recognition of the rapid advances that are occurring in chromatography, the analyst is permitted certain options to improve the separations or lower the cost of measurements. Each time such modifications are made to the method, the analyst is required to repeat the procedure in Section 8.2.

8.1.3 The laboratory must spike all samples with surrogate standards to monitor continuing laboratory performance. This procedure is described in Section 8.4.

8.2 To establish the ability to generate acceptable accuracy and precision, the analyst must perform the following operations.

8.2.1 Select a representative spike concentration for each parameter to be measured. Using stock standards, prepare a quality control check sample concentrate in acetone 1000 times more concentrated than the selected concentrations. Quality control check sample concentrates, appropriate for use with this method, will be available from the U.S Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268.

8.2.2 Using a pipet, add 1.00 mL of the check sample concentrate and 1.0 mL of the surrogate standard dosing solution (Section 6.8) to each of a minimum of four 1000-mL aliquots of reagent water. A representative wastewater may be used in place of the reagent water, but one or more additional aliquots must be analyzed to determine background levels, and the spike level must exceed twice the background level for the test to be valid. Analyze the aliquots according to the method beginning in Section 10.

8.2.3 Calculate the average percent recovery, (R), and the standard deviation of the percent recovery (s), for all parameters and surrogate standards. Wastewater background corrections must be made before R and s calculations are performed.

8.2.4 Using Table 6 or 7, note the average recovery (X) and standard deviation (p) expected for each method parameter. Compare these to the calculated values for R and s. If $s > p$ or $|X - R| > p$, review potential problem areas and repeat the test.

8.2.5 The U.S. Environmental Protection Agency plans to establish performance criteria for R and s based upon the result of interlaboratory testing. When they become available, these criteria must be met before any samples may be analyzed.

8.3 The analyst must calculate method performance criteria for each of the surrogate standards.

8.3.1 Calculate upper and lower control limits for method performance for each surrogate standard, using the values for R and s calculated in Section 8.2.3:

$$\text{Upper Control Limit (UCL)} = R + 3s$$
$$\text{Lower Control Limit (LCL)} = R - 3s$$

The UCL and LCL can be used to construct control charts(7) that are useful in observing trends in performance. The control limits above must be replaced by method performance criteria as they become available from the U.S. Environmental Protection Agency.

8.3.2 For each surrogate standard, the laboratory must develop and maintain separate accuracy statements of laboratory performance for wastewater samples. An accuracy statement for the method is defined as R ± s. The accuracy statement should be developed by the analysis of four aliquots of wastewater as described in Section 8.2.2, followed by the calculation of R and s. Alternately, the analyst may use four wastewater data points gathered through the requirement for continuing quality control in Section 8.4. The accuracy statements should be updated regularly.(7)

8.4 The laboratory is required to spike all samples with the surrogate standard spiking solution to monitor spike recoveries. If the recovery for any surrogate standard does not fall within the control limits for method performance, the results reported for that sample must be qualified as described in Section 15.3. The laboratory should monitor the frequency of data so qualified to ensure that it remains at or below 5%.

8.5 Before processing any samples, the analyst should demonstrate through the analysis of a one-liter aliquot of reagent water, that all glassware and reagent interferences are under control. Each time a set of samples is extracted or there is a change in reagents, a laboratory reagent blank should be processed as a safeguard against laboratory contamination.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. Whenever possible, the laboratory should perform analysis of standard reference materials and participate in relevant performance evaluation studies.

9. Sample Collection, Preservation, and Handling

9.1 Grab samples must be collected in glass containers. Conventional sampling practices(8) should be followed, except that the bottle must not be prewashed with sample before collection. Composite samples should be collected in refrigerated glass containers in accordance with the requirements of the program. Automatic sampling equipment must be as free as possible of Tygon and other potential sources of contamination.

9.2 The samples must be iced or refrigerated at 4 °C from the time of collection until extraction. Fill the sample bottles and, if residual chlorine is present, add 80 mg of sodium thiosulfate per each liter of water. U.S. Environmental Protection Agency methods 330.4 and 330.5 may be used to measure the residual chlorine(9). Field test kits are available for this purpose.

9.3 All samples must be extracted within 7 days and completely analyzed within 40 days of extraction.

10. Separatory Funnel Extraction

10.1 Samples are usually extracted using separatory funnel techniques. If emulsions will prevent achieving acceptable solvent recovery with separatory funnel extractions, continuous extraction (Section 11) may be used. The separatory funnel extraction scheme described below assumes a sample volume of one-liter. When sample volumes of two liters are to be extracted, use 250-, 100-, and 100-mL volumes of methylene chloride for the serial extraction of the base/neutrals and 200-, 100-, and 100-mL volumes of methylene chloride for the acids.

10.2 Mark the water meniscus on the side of the sample bottle for later determination of sample volume. Pour the entire sample into a two-liter separatory funnel. Pipet 1.00 mL surrogate standard spiking solution into the separatory funnel and mix well. Check the pH of the sample with wide-range pH paper and adjust to pH > 11 with 10 N sodium hydroxide.

10.3 Add 60 mL methylene chloride to the sample bottle, seal, and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for two minutes with periodic venting to release excess pressure. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. The optimum technique depends upon the sample, but may include stirring, filtration of the emulsion through glass wool, centrifugation, or other physical methods. Collect the methylene chloride extract in a 250-ml Erlenmeyer flask. If the emulsion cannot be broken (recovery of less than 80% of the methylene chloride, corrected for the water solubility of methylene chloride), transfer the sample, solvent, and emulsion into the extraction chamber of a continuous extractor and proceed as described in Section 11.3.

10.4 Add a second 60-mL volume of methylene chloride to the sample bottle and repeat the extraction procedure a second time, combining the extracts in the Erlenmeyer flask. Perform a third extraction in the same manner. Label the combined extract as base/neutral fraction.

10.5 Adjust the pH of the aqueous phase to less than 2 using sulfuric acid (1 + 1). Serially extract three times with 60-mL aliquots of methylene chloride. Collect and combine the extracts in a 250-mL Erlenmeyer flask and label the combined extract as the acid fraction.

10.6 For each fraction, assemble a Kuderna-Danish (K-D) concentrator by attaching a 10-mL concentrator tube to a 500-mL evaporative flask. Other concentration devices or techniques may be used in place of the K-D if the requirements of Section 8.2 are met.

10.7 For each fraction, pour the combined extract through a drying column containing about 10 cm of anhydrous sodium sulfate, and collect the extract in the K-D concentrator. Rinse the Erlenmeyer flask and column with 20 to 30 mL of methylene chloride to complete the quantitative transfer.

10.8 To the evaporative flask for each fraction, add one or two clean boiling chips and attach a three-ball Snyder column. Prewet the Snyder column by adding about 1 mL methylene chloride to the top of the column. Place the K-D apparatus on a hot water bath (60 ° to 65 °C) so that the concentrator tube is partially immersed in the hot water, and the entire lower rounded surface of the flask is bathed with hot vapor. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 15 to 20 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches 1 mL, remove the K-D apparatus from the water bath and allow it to drain and cool for at least 10 minutes. Remove the Snyder column and rinse the flask and its lower joint into the concentrator tube with 1 to 2 mL of methylene chloride. A 5-mL syringe is recommended for this operation.

10.9 Add another one or two clean boiling chips to the concentrator tube and attach a two-ball micro Snyder column. Prewet the Snyder column by adding about 0.5 mL of methylene chloride to the top of the column. Place the K-D apparatus on a hot water bath (60° to 65 °C) so that the concentrator tube is partially immersed in the hot water. Adjust the vertical position of the apparatus and the water temperature as required to complete the concentration in 5 to 10 minutes. At the proper rate of distillation the balls of the column will actively chatter but the chambers will not flood with condensed solvent. When the apparent volume of liquid reaches about 0.5 mL, remove the K-D apperatus from the water bath and allow it to drain for at least 10 minutes while cooling. Remove the Snyder column and rinse its the flask and its lower joint into the concentrator tube with 0.2 mL of acetone or methylene chloride. Adjust the final volume to 1.0 mL with the solvent. Stopper the concentrator tube and store refrigerated if GC/MS analysis will not be performed immediately. If the extracts will be stored longer than two days, they should be transferred to Teflon-sealed screw-cap bottles and labeled base/neutral or acid fraction as appropriate.

10.10 Determine the original sample volume by refilling the sample bottle to the mark and transferring the water to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11. Continuous Extraction

11.1 When experience with a sample from a given source indicates that a serious emulsion problem will result or an emulsion is encountered in Section 10.3, using a separatory funnel, a continuous extractor should be used.

11.2 Mark the water meniscus on the side of the sample bottle for later measurement of the sample volume. Check the pH of the sample with wide-range pH paper and adjust to pH 11 with 10 N sodium hydroxide. Transfer the sample to the continuous extractor and using a pipet, add 1.00 mL of surrogate standard spiking solution and mix well. Add 60 mL of methylene chloride to the sample bottle, seal and shake for 30 seconds to rinse the inner surface. Transfer the solvent to the extractor.

11.3 Repeat the sample bottle rinse with an additional 50- to 100-mL portion of methylene chloride and add the rinse to the extractor.

11.4 Add 200 to 500 mL of methylene chloride to the distilling flask, add sufficient reagent water to ensure proper operation, and extract for 24 hours. Allow to cool, then detach the boiling flask, and dry, concentrate and seal the extract as in Section 10.6 through 10.9. Hold the aqueous phase for acid extraction (See Section 11.5).

11.5 Charge a clean distilling flask with 500 mL of methylene chloride and attach it to the continuous extractor. Carefully, adjust the pH of the aqueous phase to less than 2 using sulfuric acid (1 + 1). Extract for 24 hours. Dry, concentrate and label and seal the extract as described in Sections 10.6 through 10.9.

12. Daily GC/MS Performance Tests

12.1 At the beginning of each day that analyses are to be performed, the GC/MS system must be checked to see that acceptable performance criteria are achieved for DFTPP. Each day that benzidine is to be determined, the tailing factor criterion described in Section 12.4 must be achieved. Each day the acids are to be determined, the tailing factor criterion in Section 12.5 must be achieved.

12.2 These DFTPP performance test require the following instrumental parameters.

Electron Energy	70 volts (nominal)
Mass Range	35 to 450 amu
Scan Time	to give at least 5 scans per peak but not to exceed 7 seconds per scan.

12.3 DFTPP performance test(10)—At the beginning of each day, inject 2μL (50 ng) of DFTPP standard solution. Obtain a background corrected mass spectra of DFTPP and check that all the key ion criteria in Table 9 are achieved. If all the criteria are not achieved, the analyst must retune the mass spectrometer and repeat the test until all criteria are achieved. The performance criteria must be achieved before any samples, blanks, or standards are analyzed. The tailing factor tests in Section 12.4 and 12.5 may be performed simultaneously with the test.

12.4 Column performance test for base/neutrals—At the beginning of each day that the base-neutral fraction is to be analyzed for benzidine, the benzidine tailing factor must be calculated. Inject 100 ng of benzidine either separately or as a part of a standard mixture that may contain DFTPP and calculate the tailing factor. The benzidine tailing factor must be less than 3.0. Calculation of the tailing factor is illustrated in Figure 13.(11) Replace the column packing if the tailing factor criterion cannot be achieved.

12.5 Column performance for acids—At the beginning of each day that the acids are to be determined, inject 50 ng of pentachlorophenol either separately or as a part of a standard mix that may contain DFTPP. The tailing factor for pentachlorophenol must be less than five. Calculation of the tailing factor is illustrated in Figure 13(11). Replace the column packing if the tailing factor criterion cannot be achieved.

13. Gas Chromatography/ Mass Spectrometry

13.1 Table 4 summarizes the recommended gas chromatographic operating conditions for the base/neutral fraction. Table 5 summarizes the recommended gas chromatographic operating conditions for determination of the acid fraction. These tables include retention times and MDL that were achieved under these conditions. Examples of the parameter separations achieved by these columns are shown in Figures 1 through 12. Other packed columns or chromatographic conditions may be used if the requirements of Section 8.2 and Section 12 are met. Capillary (open-tubular) columns may also be used if the relative standard deviations of responses for replicate injections are demonstrated to be less than 6% and the requirements of Section 8.2 and Section 12 are met.

13.2 After the GC/MS performance requirements of Section 12, calibrate the system daily as described in Section 7.

13.3 If the internal standard approach is being used, the internal standard must be added to sample extract and mixed thoroughly, immediately, before injection into the instrument. This minimizes losses due to adsorption, chemical reaction or evaporation.

13.4 Inject 2 to 5 μL of the sample extract using the solvent-flush technique(12). Smaller (1.0 µL) volumes may be injected if automatic devices are employed. Record the volume injected to the nearest 0.05 µL.

13.5 If the response for any ion exceeds the working range of the GC/MS system, dilute the extract and reanalyze.

13.6 Perform all qualitative and quantitative measurements as described in Sections 14 and 15. When the extracts are not being used for analyses, store them at 4 °C protected from light in screw-cap vials equipped with unpierced Teflon-lined septa.

14. Qualitative Identification

14.1 Obtain an EICP for the primary ion and the two other ions listed in Tables 4 and 5. See Section 7.3 for ions to be used with internal and surrogate standards. The following criteria must be met to make a qualitative identification.

14.1.1 The characteristic ions for each compound of interest must maximize in the same or within one scan of each other.

14.1.2 The retention time must fall within ± 30 seconds of the retention time of the authentic compound.

14.1.3 The relative peak heights of the three characteristic ions in the EICP's must fall within ± 20% of the relative intensities of these ions in a reference mass spectrum. The reference mass spectrum can be obtained by a standard analyzed in the GC/MS system or from a reference library.

14.2 Structural isomers that have very similar mass spectra and less than 30 seconds difference in retention time, can be explicitly identified only if the resolution between authentic isomers in a standard mix is acceptable. Acceptable resolution is achieved if the baseline to valley height between the isomers is less than 25% of the sum of the two peak heights. Otherwise, structural isomers are identified as isomeric pairs.

15. Calculations

15.1 When a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion in Tables 4 and 5. Use the base peak ion for internal and surrogate standards. If the sample produces an interference for the first listed ion, use a secondary ion to quantitate. Quantitation will be performed using external or internal standard techniques.

15.1.1 If the external standard calibration procedure is used, calculate the amount of material injected from the area of the characteristic ion using the calibration curve or calibration factor in Section 7.2.2. The concentration in the sample can be calculated from equation 2:

$$\text{Eq. 2.}\quad \text{Concentration, } \mu g/L = \frac{(A)(V_t)}{(V_i)(V_s)}$$

where:

A = Amount of material injected, in nanograms.

V_i = Volume of extract injected (µL).

V_t = Volume of total extract (µL).

V_s = Volume of water extracted (mL).

15.1.2 If the internal standard calibration procedure was used, calculate the concentration in the sample using the response factor (RF) determined in Section 7.3.2 and equation 3.

Eq. 3

$$\text{Concentration, } \mu g/L = \frac{(A_s)(I_s)}{(A_{is})(RF)(V_o)}$$

where:

A_s = Area of the characteristic ion for the parameter to be measured.

A_{is} = Area of the characteristic ion for the internal standard.

I_s = Amount of internal standard added to each extract (µg).

V_o = Volume of water extracted (liters).

15.2 Report results in micrograms per liter without correction for recovery data. When duplicate and spiked samples are analyzed, report all data obtained with the sample results.

15.3 If the surrogate standard recovery falls outside the control limits in Section 8.3, data for all parameters in that fraction of the sample must be labeled as suspect.

16. Method Performance

16.1 The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with a 99% confidence that the value is above zero(1). The MDL concentrations listed in Tables 4 and 5 were obtained using reagent water(13).

16.2 The average recoveries and the average standard deviations of the percent recoveries, presented in Table 5, were the result of a study of the accuracy and precision of this method by several laboratories. The values listed represent the results from two to four laboratories(14).

16.3 The U.S. Environmental Protection Agency is in the process of conducting an interlaboratory method study to fully define the performance of this method.

17. Screening Procedure for 2,3,7,8-TCDD

17.1 If the sample must be screened for the presence of 2,3,7,8-TCDD, it is recommended that the reference material not be handled in the laboratory unless extensive safety precautions are employed. It is sufficient to analyze the base/neutral extract by selected ion monitoring (SIM) GC/MS techniques, as follows:

17.1.1 Concentrate the base/neutral extract to a final volume of 0.2 mL.

17.1.2 Adjust the temperature of the base/neutral column (Section 5.6.2) to 220 °C.

17.1.3 Operate the mass spectrometer to acquire data in the SIM mode using the ions at m/e 257, 320 and 322 and a dwell time no greater than 333 milliseconds per ion.

17.1.4 Inject 5 to 7 µL of the base/neutral extract. Collect SIM data for a total of 10 minutes.

17.1.5 The possible presence of 2,3,7,8-TCDD is indicated if all three ions exhibit simultaneous peaks at any point in the selected ion current profiles.

17.1.6 For each occurrence where the possible presence of 2,3,7,8-TCDD is indicated, calculate and retain the relative abundances of each of the three ions.

17.2 False positives to this test may be caused by the presence of single or coeluting combinations of compounds whose mass spectra contain all of these ions.

17.3 Conclusive results of the presence and concentration level of 2,3,7,8-TCDD can be obtained only from a properly equipped laboratory through the use of method 613 of other approved alternate test procedures.

References

1. See Appendix A.

2. "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants." U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1977, Revised April 1977. Available from Effluent Guidelines Division, Washington, DC 20460.

3. ASTM Annual Book of Standards, Part 31, D 3694. "Standard Practice for Preparation of Sample Containers and for Preservation," American Society for Testing and Materials, Philadelphia, PA, p. 679, (1980).

4. "Carcinogens—Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

5. "OSHA Safety and Health Standards, General Industry," (29CFR1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

6. "Safety in Academic Chemistry Laboratories," American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

7. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

8. ASTM Annual Book of Standards, Part 31, D 3370, "Standard Practice for Sampling Water," American Society for Testing and Materials, Philadelphia, PA, p. 76, 1980.

9. "Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPD) for Chlorine, Total Residual," Methods for Chemical Analysis of Water and Wastes, EPA 600-4/79-020, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979.

10. Eichelberger, J.W., Harris, L.E., and Budde, W.L., "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography-Mass Spectrometry," *Analytical Chemistry*, 47, 995 (1975).

11. McNair, H.M. and Bonelli, E.J., "Basic Chromatography," Consolidated Printing, Berkeley, California, p. 52, 1969.

12. Burke, J.A., "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects," *Journal of the Association of Official Analytical Chemists, 48,* 1037 (1965).

13. "Method Detection Limit for Methods 624 and 625," Olynyk, P., Budde, W.L., Eichelberger, J.W., unpublished report October, 1980.

14. Kloepfer, R.D., "POTW Toxic Study, Analytical Quality Assurance Final Report," U.S. Environmental Protection Agency, Region VII, Kansas City, Kansas 66115, 1981.

Table 1. *Base/Neutral Extractables*

Parameter	STORET No.	CAS No.
Acenaphthene	34205	83-32-9
Acenaphthylene	34200	208-96-8
Anthracene	34220	120-12-7
Aldrin	39330	309-00-2
Benzo(a)anthracene	34526	56-55-3
Benzo(b)fluoranthene	34230	205-99-2
Benzo(k)fluoranthene	34242	207-08-9
Benzo(a)pyrene	34247	50-32-8
Benzo(ghi)perylene	34521	191-24-2
Benzyl butyl phthalate	34292	85-68-7
β-BHC	39338	319-85-7
δ-BHC	34259	319-86-8
Bis(2-chloroethyl)ether	34273	111-44-4
Bis(2-chloroethoxy)methane	34278	111-91-1
Bis(2-ethylhexyl)phthalate	39100	117-81-7
Bis(2-chloroisopropyl)ether	34283	108-60-1
4-Bromophenyl phenyl ether	34636	101-55-3
Chlordane	39350	57-74-9
2-Chloronaphthalene	34581	91-58-7
4-Chlorophenyl phenyl ether	34641	7005-72-3
Chrysene	34320	218-01-9
4,4'-DDD	39310	72-54-8
4,4'-DDE	39320	72-55-9
4,4'-DDT	39300	50-29-3
Dibenzo(a,h)anthracene	34556	53-70-3
Di-n-butylphthalate	39110	84-74-2
1,3-Dichlorobenzene	34566	541-73-1
1,2-Dichlorobenzene	34536	95-50-1
1,4-Dichlorobenzene	34571	106-46-7
3,3'-Dichlorobenzidine	34631	91-94-1
Dieldrin	39380	60-57-1
Diethyl phthalate	34336	84-66-2
Dimethyl phthalate	34341	131-11-3
2,4-Dinitrotoluene	34611	121-14-2
2,6-Dinitrotoluene	34626	606-20-2
Di-n-octylphthalate	34596	117-84-0
Endosulfan sulfate	34351	1031-07-8
Endrin aldehyde	34366	7421-93-4
Fluoranthene	34376	206-44-0
Fluorene	34381	86-73-7
Heptachlor	39410	76-44-8
Heptachlor epoxide	39420	1024-57-3
Hexachlorobenzene	39700	118-74-1
Hexachlorobutadiene	34391	87-68-3
Hexachloroethane	34396	67-72-1
Indeno(1,2,3-cd)pyrene	34403	193-39-5
Isophorone	34408	78-59-1
Naphthalene	34696	91-20-3
Nitrobenzene	34447	98-95-3
N-Nitrosodi-n-propylamine	34428	621-64-7
PCB-1016	34671	12674-11-2
PCB-1221	39488	11104-28-2
PCB-1232	39492	11141-16-5
PCB-1242	39496	53469-21-9
PCB-1248	39500	12672-29-6
PCB-1254	39504	11097-69-1
PCB-1260	39508	11096-82-5
Phenanthrene	34461	85-01-8
Pyrene	34469	129-00-0
Toxaphene	39400	8001-35-2
1,2,4-Trichlorobenzene	34551	120-82-1

Table 2. Acid Extractables

Parameter	STORET No.	CAS No.
4-Chloro-3-methylphenol	34452	59-50-7
2-Chlorophenol	34586	95-57-8
2,4-Dichlorophenol	34601	120-83-2
2,4-Dimethylphenol	34606	105-67-9
2,4-Dinitrophenol	34616	51-28-5
2-Methyl-4,6-dinitrophenol	34657	534-52-1
2-Nitrophenol	34591	88-75-5
4-Nitrophenol	34646	100-02-7
Pentachlorophenol	39032	87-86-5
Phenol	34694	108-95-2
2,4,6-Trichlorophenol	34621	88-06-2

Table 3. Additional Extractable Parameters[a]

Parameter	STORET No.	CAS No.	Method
Benzidine	39120	92-87-5	605
α-BHC	39337	319-84-6	608
γ-BHC	39340	58-89-8	608
Endosulfan I	34361	959-98-8	608
Endosulfan II	34356	33213-65-9	608
Endrin	39390	72-20-8	608
Hexachlorocyclopentadiene	34386	77-47-4	612
N-Nitrosodimethylamine	34438	62-75-9	605
N-Nitrosodiphenylamine	34433	86-30-6	605

[a] See Section 1.2 of method

Table 4. Chromatographic Conditions, Method Detection Limits and Characteristic Ions for Base/Neutral Extractables

			Characteristic Ions					
	Retention Time	Method Detection	Electron Impact			Chemical Ionization (Methane)		
Parameter	(min.)	Limit (μg/L)	Primary	Secondary				
1,3-Dichlorobenzene	7.4	1.9	146	148	113	146	148	150
1,4-Dichlorobenzene	7.8	4.4	146	148	113	146	148	150
Hexachloroethane	8.4	1.6	117	201	199	199	201	203
Bis(2-chloroethyl)ether	8.4	5.7	93	63	95	63	107	109
1,2-Dichlorobenzene	8.4	1.9	146	148	113	146	148	150
Bis(2-chloroisopropyl)ether	9.3	5.7	45	77	79	77	135	137
N-Nitrosodi-n-propyl amine			130	42	101			
Nitrobenzene	11.1	1.9	77	123	65	124	152	164
Hexachlorobutadiene	11.4	0.9	225	223	227	223	225	227
1,2,4-Trichlorobenzene	11.6	1.9	180	182	145	181	183	209
Isophorone	11.9	2.2	82	95	138	139	167	178
Naphthalene	12.1	1.6	128	129	127	129	157	169
Bis(2-chloroethoxy)methane	12.2	5.3	93	95	123	65	107	137
Hexachlorocyclopentadiene*	13.9		237	235	272	235	237	239
2-Chloronaphthalene	15.9	1.9	162	164	127	163	191	203
Acenaphthylene	17.4	3.5	152	151	153	152	153	181
Acenaphthene	17.8	1.9	154	153	152	154	155	183
Dimethyl phthalate	18.3	1.6	163	194	164	151	163	164
2,6-Dinitrotoluene	18.7	1.9	165	89	121	183	211	223
Fluorene	19.5	1.9	166	165	167	166	167	195
4-Chlorophenyl phenyl ether	19.5	4.2	204	206	141			
2,4-Dinitrotoluene	19.8	5.7	165	63	182	183	211	223
Diethylphthalate	20.1	22	149	177	150	177	223	251
N-Nitrosodiphenylamine*	20.5	1.9	169	168	167	169	170	198
Hexachlorobenzene	21.0	1.9	284	142	249	284	286	288
α-BHC*	21.1		183	181	109			
4-Bromophenyl phenyl ether	21.2	1.9	248	250	141	249	251	277
γ-BHC*	22.4		183	181	109			
Phenanthrene	22.8	5.4	178	179	176	178	179	207
Anthracene	22.8	1.9	178	179	176	178	179	207
β-BHC	23.4	4.2	181	183	109			
Heptachlor	23.4	1.9	100	272	274			
δ-BHC	23.7	3.1	183	109	181			
Aldrin	24.0	1.9	66	263	220			

Table 4. *(Continued)*

Parameter	Retention Time (min.)	Method Detection Limit (µg/L)	Characteristic Ions					
			Electron Impact			Chemical Ionization (Methane)		
			Primary	Secondary				
Dibutyl phthalate	24.7	2.5	149	150	104	149	205	279
Heptachlor epoxide	25.6	2.2	353	355	351			
Endosulfan I*	26.4	—	237	339	341			
Fluoranthene	26.5	2.2	202	101	100	203	231	243
Dieldrin	27.2	2.5	79	263	279			
4,4'-DDE	27.2	5.6	246	248	176			
Pyrene	27.3	1.9	202	101	100	203	231	243
Endrin*	27.9	—	81	263	82			
Endosulfan II*	28.6	—	237	339	341			
4,4'-DDD	28.6	2.8	235	237	165			
Benzidine*	28.8	44	184	92	185	185	213	225
4,4'-DDT	29.3	4.7	235	237	165			
Endosulfan sulfate	29.8	5.6	272	387	422			
Endrin aldehyde	—	—	67	345	250			
Butyl benzyl phthalate	29.9	2.5	149	91	206	149	299	327
Bis(2-ethylhexyl) phthalate	30.6	2.5	149	167	279	149		
Chrysene	31.5	2.5	228	226	229	228	229	257
Benzo(a)anthracene	31.5	7.8	228	229	226	228	229	257
3,3'-Dichlorobenzidine	32.2	16.5	252	254	126			
Di-n-octylphthalate	32.5	2.5	149					
Benzo(b)fluoranthene	34.9	4.8	252	253	125	252	253	281
Benzo(k)fluoranthene	34.9	2.5	252	253	125	252	253	281
Benzo(a)pyrene	36.4	2.5	252	253	125	252	253	281
Indeno(1,2,3-c,d)pyrene	42.7	3.7	276	138	277	276	277	305
Dibenzo(a,h)anthracene	43.2	2.5	278	139	279	278	279	307
Benzo(ghi)perylene	45.1	4.1	276	138	277	276	277	305
N-Nitrosodimethyl amine*	—	—	42	74	44			
Chlordane**	19 to 30	—	373	375	377			
Toxaphene**	25 to 34	—	159	231	233			
PCB 1016**	18 to 30	—	224	260	294			
PCB 1221**	15 to 30	30	190	224	260			
PCB 1232**	15 to 32	—	190	224	260			
PCB 1242**	15 to 32	—	224	260	294			
PCB 1248**	12 to 34	—	294	330	362			
PCB 1254**	22 to 34	36	294	330	362			
PCB 1260**	23 to 32	—	330	362	394			

*See Section 1.2.

**These compounds are mixtures of various isomers. (See Figures 2 to 12)

Gas Chromatographic conditions: Glass column 1.8 m long × 2 mm ID packed with Supelcoport (100/120 mesh) coated with 3% SP-2250. Carrier gas: helium at a flow rate of 30 mL per min.
Temperature: Isothermal at 50°C for 4 min., then 8° per min to 270°C. Hold at 270°C for 30 min.

Table 5. Chromatographic Conditions, Method Detection Limits and Characteristic Ions for Acid Extractables

Parameter	Retention Time (min.)	Method Detection Limit (µg/L)	Characteristic Ions					
			Electron Impact			Chemical Ionization (Methane)		
			Primary	Secondary				
2-Chlorophenol	5.9	3.3	128	64	130	129	131	157
2-Nitrophenol	6.5	3.6	139	65	109	140	168	122
Phenol	8.0	1.5	94	65	66	95	123	135
2,4-Dimethylphenol	9.4	2.7	122	107	121	123	151	163
2,4-Dichlorophenol	9.8	2.7	162	164	98	163	165	167
2,4,6-Trichlorophenol	11.8	2.7	196	198	200	197	199	201
4-Chloro-3-methylphenol	13.2	3.0	142	107	144	143	171	183
2,4-Dinitrophenol	15.9	42	184	63	154	185	213	225
2-Methyl-4,6-dinitrophenol	16.2	24	198	182	77	199	227	239
Pentachlorophenol	17.5	3.6	266	264	268	267	265	269
4-Nitrophenol	20.3	2.4	65	139	109	140	168	122

Chromatographic conditions: 1.8 m long × 2 mm ID glass column packed with Supelcoport (100/120 mesh) coated with 1% SP-1240. Carrier gas: helium at a flow rate of 30 mL per min. Column temperature, isothermal at 70°C for 2 min, then 8° per min, to 200°.

Table 6. Accuracy and Precision for Base/Neutral Extractables

	Reagent Water		Wastewater	
Parameter	Average Percent Recovery	Standard Deviation (%)	Average Percent Recovery	Standard Deviation (%)
Acenaphthene	77	23	83	29
Acenaphthylene	78	22	82	23
Aldrin	72	6	—	—
Anthracene	84	14	76	22
Benzo(a)anthracene	83	19	75	28
Benzo(b)fluoranthene	96	68	41	21
Benzo(k)fluoranthene	96	68	47	27
Benzo(ghi)perylene	80	45	68	40
Benzo(a)pyrene	90	22	43	21
Benzidine	87	61	63	55
Butyl benzyl phthalate	47	32	74	43
β-BHC	69	25	—	—
δ-BHC	56	18	—	—
Bis(2-chloroethoxy)methane	84	33	82	74
Bis(2-chloroethyl)ether	56	36	72	37
Bis(2-chloroisopropyl)ether	71	33	71	39
Bis (2-ethylhexyl) phthalate	129	50	82	63
4-Bromophenyl phenyl ether	80	17	75	20
2-Chloronaphthalene	73	24	79	27
4-Chlorophenyl phenyl ether	45	11	—	—
Chrysene	83	19	75	28
4,4′-DDD	80	9	—	—
4,4′-DDE	69	20	—	—
4,4′-DDT	63	15	—	—
Dibenzo(a,h)anthracene	82	39	70	40
Di-n-butyl phthalate	70	25	93	51
1,2-Dichlorobenzene	59	27	62	28
1,3-Dichlorobenzene	55	28	54	24
1,4-Dichlorobenzene	61	31	63	35
3,3′-Dichlorobenzidine	184	174	143	145
Diethylphthalate	42	28	48	28
Dimethyl phthalate	25	33	35	36
2,4-Dinitrotoluene	83	32	79	34
2,6-Dinitrotoluene	79	18	79	25
Di-n-octylphthalate	97	37	89	62
Endosulfan sulfate	79	29	—	—
Fluoranthene	89	19	80	26
Fluorene	77	16	80	20
Heptachlor	69	6	—	—
Heptachlor epoxide	82	7	—	—
Hexachlorobenzene	79	20	71	22
Hexachlorobutadiene	46	25	48	28
Hexachlorocyclopentadiene	27	25	12	12
Hexachloroethane	46	21	52	26
Indeno (1,2,3-cd) pyrene	65	37	81	43
Isophorone	75	33	77	42
Naphthalene	6	32	75	35
Nitrobenzene	72	31	82	54
N-Nitrosodi-n-propylamine	68	39	76	45
N-Nitrosodiphenylamine	84	24	86	31
PCB-1221	77	11	—	—
PCB-1254	80	13	—	—
Phenanthrene	84	14	76	22
Pyrene	86	15	80	23
1,2,4-Trichlorobenzene	64	16	69	26

Spiked between 5 to 2400 μg/L.

Table 7. *Accuracy and Precision for Acid Extractables*

Parameter	Reagent Water Average Percent Recovery	Reagent Water Standard Deviation (%)	Wastewater Average Percent Recovery	Wastewater Standard Deviation (%)
4-Chloro-3-methylphenol	79	18	75	21
2-Chlorophenol	70	23	71	25
2,4-Dichlorophenol	74	24	80	21
2,4-Dimethylphenol	64	25	58	26
2,4-Dinitrophenol	78	21	108	56
2-Methyl-4,6-dinitrophenol	83	18	90	35
4-Nitrophenol	41	20	43	16
2-Nitrophenol	75	25	75	27
Pentachlorophenol	86	20	66	36
Phenol	36	14	36	21
2,4,6-Trichlorophenol	77	20	81	20

Spiked from 10 to 1500 μg/L.

Table 8. *Suggested Internal and Surrogate Standards*

Base/Neutral Fraction	Acid Fraction
Aniline-d_5	2-Fluorophenol
Anthracene-d_{10}	Pentafluorophenol
Benzo(a)anthracene-d_{12}	Phenol-d_5
4,4'-Dibromobiphenyl	2-Perfluoromethyl phenol
4,4'-Dibromooctafluorobiphenyl	
Decafluorobiphenyl	
2,2'-Difluorobiphenyl	
4-Fluoroaniline	
1-Fluoronaphthylene	
2-Fluoronaphthylene	
Naphthalene-d_8	
Nitrobenzene-d_5	
2,3,4,5,6-Pentafluorobiphenyl	
Phenanthrene-d_{10}	
Pyridine-d_5	

Table 9. *DFTPP Key Ions and Ion Abundance Criteria*

Mass	Ion Abundance Criteria
51	30-60% of mass 198
68	less than 2% of mass 69
70	less than 2% of mass 69
127	40-60% of mass 198
197	less than 1% of mass 198
198	base peak, 100% relative abundance
199	5-9% of mass 198
275	10-30% of mass 198
365	greater than 1% of mass 198
441	present but less than mass 443
442	greater than 40% of mass 198
443	17-23% of mass 442

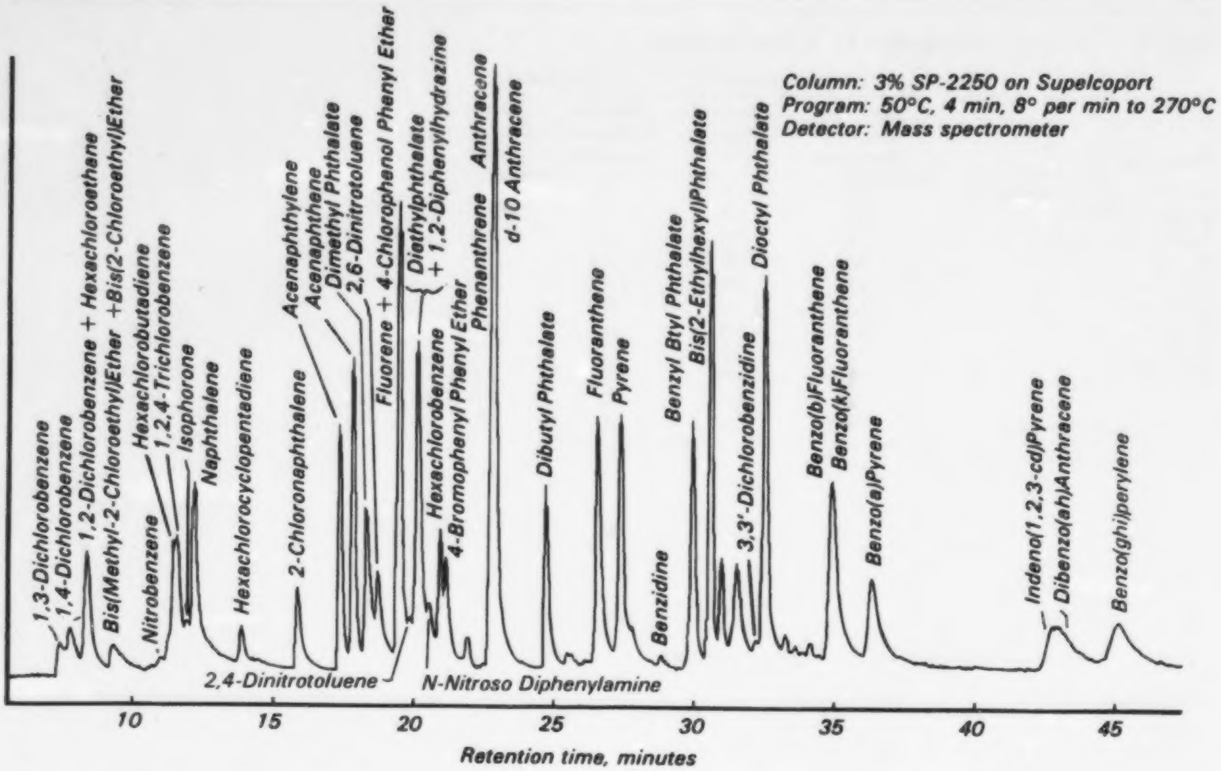


Figure 1. *Gas chroamatogram of base/neutral fraction.*

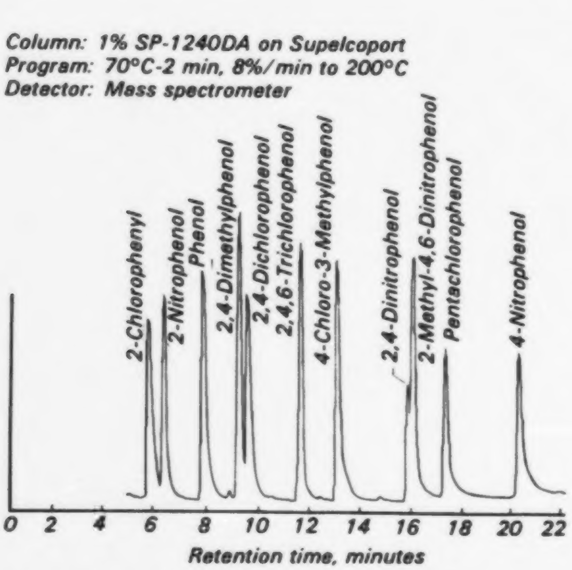


Figure 2. *Gas chromatogram of acid fraction.*

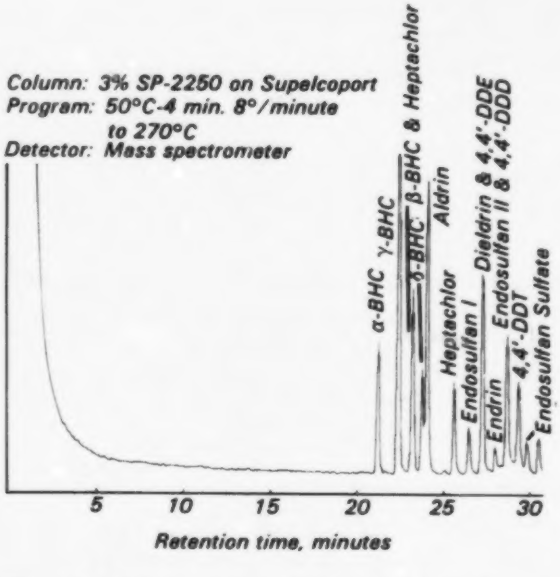


Figure 3. *Gas chromatogram of pesticide fraction.*

Column: 3% SP-2250 on Supelcoport
Program: 50°C. 4 min., 8° per min. to 270°C.
Detector: Mass spectrometer

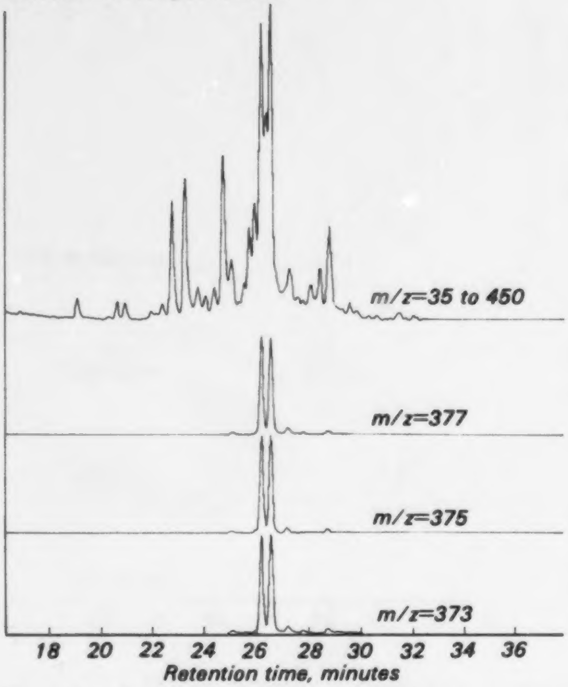


Figure 4. *Gas chromatogram of chlordane.*

Column: 3% SP-2250 on Supelcoport
Program: 50°C. 4 min., 8° per min. to 270°C.
Detector: Mass spectrometer

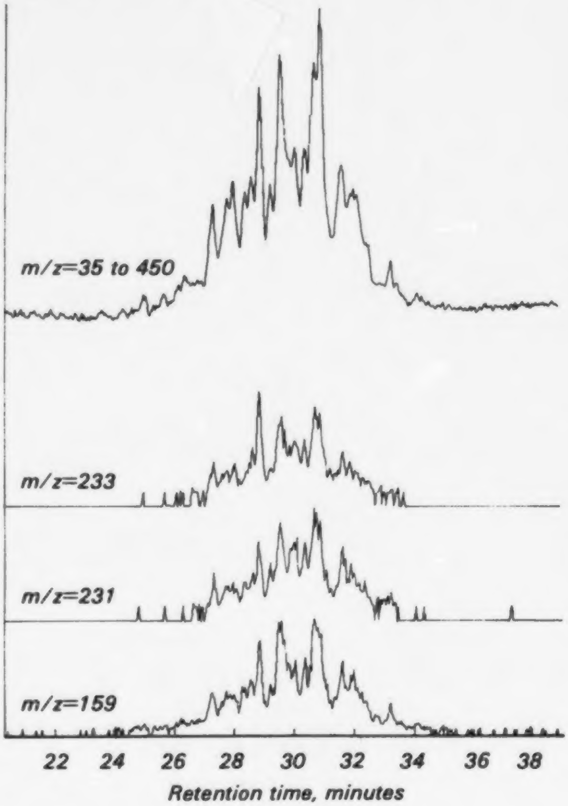


Figure 5. *Gas chromatogram of toxaphene.*

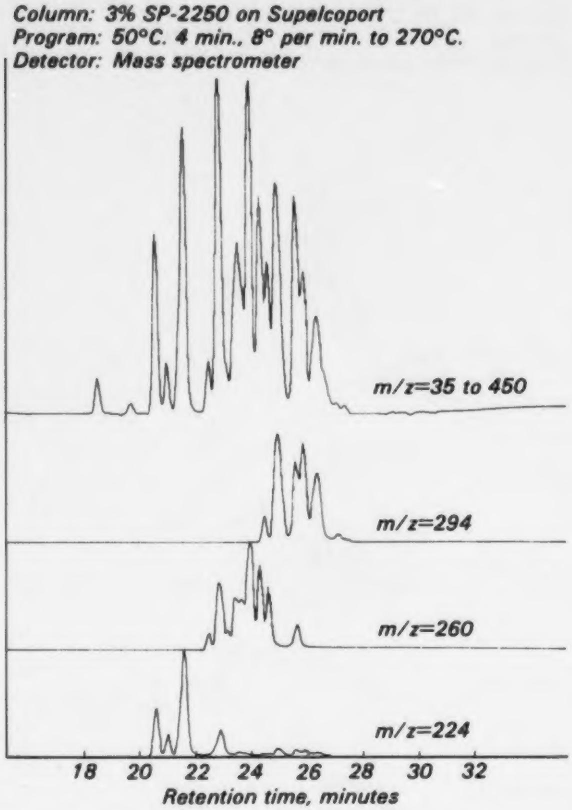


Figure 6. *Gas chromatogram of PCB-1016.*

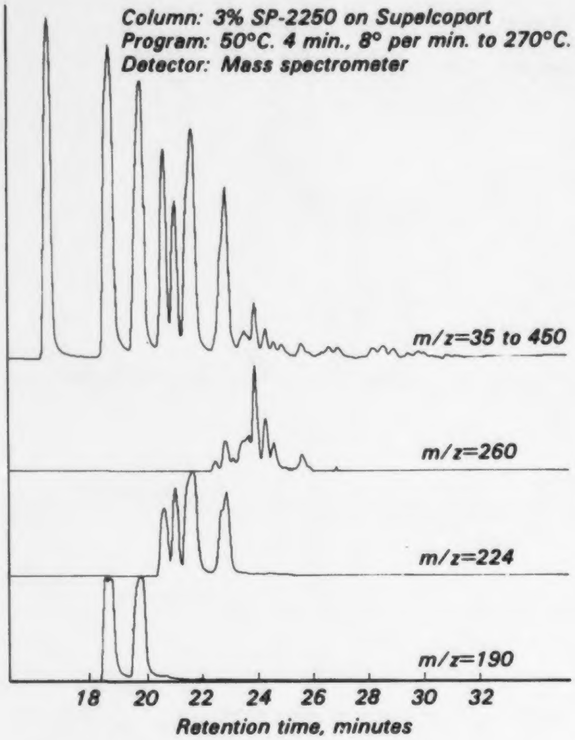
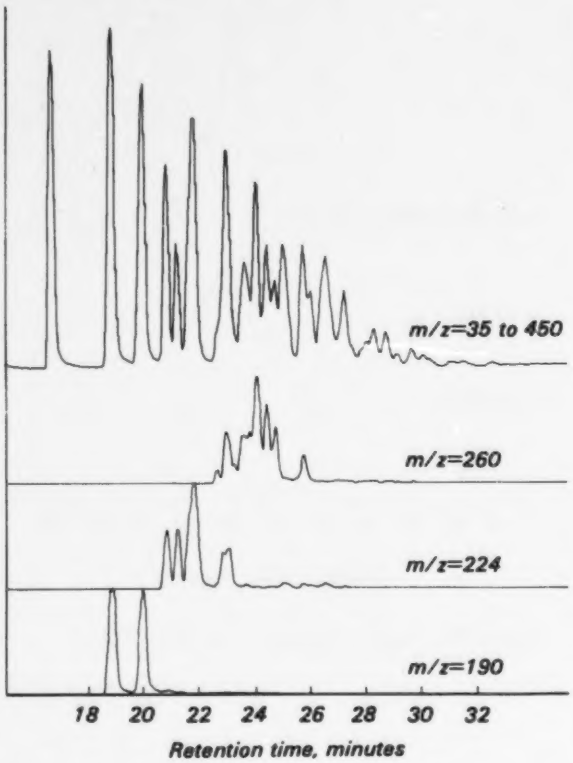
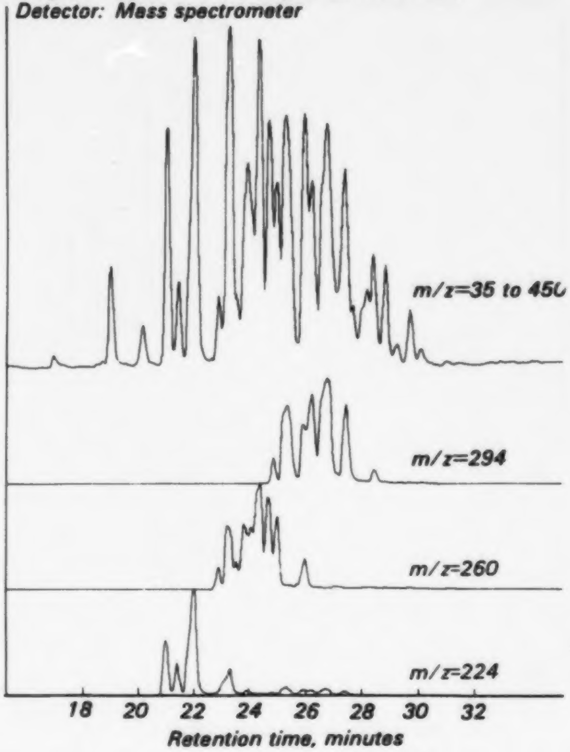


Figure 7. *Gas chromatogram of PCB-1221.*



Column: 3% SP-2250 on Supelcoport
Program: 50°C. 4 min., 8° per min. to 270°C.
Detector: Mass spectrometer

Figure 8. *Gas chromatogram of PCB-1232.*



Column: 3% SP-2250 on Supelcoport
Program: 50°C. 4 min., 8° per min. to 270°C.
Detector: Mass spectrometer

Figure 9. *Gas chromatogram of PCB-1242.*

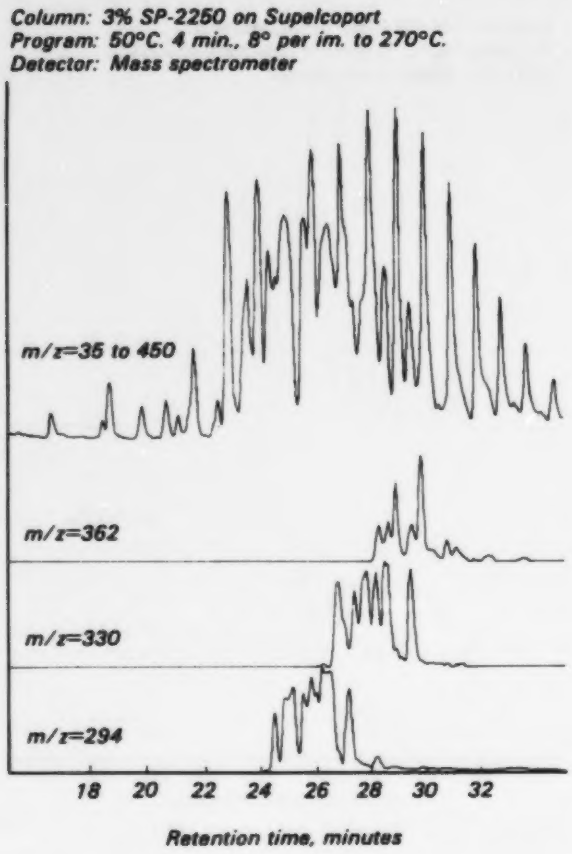


Figure 10. *Gas chromatogram of PCB-1248.*

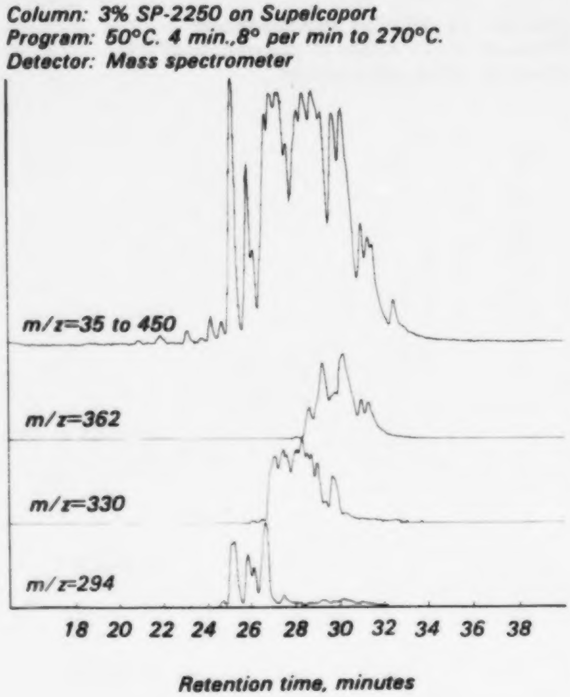


Figure 11. *Gas chromatogram of PCB-1254.*

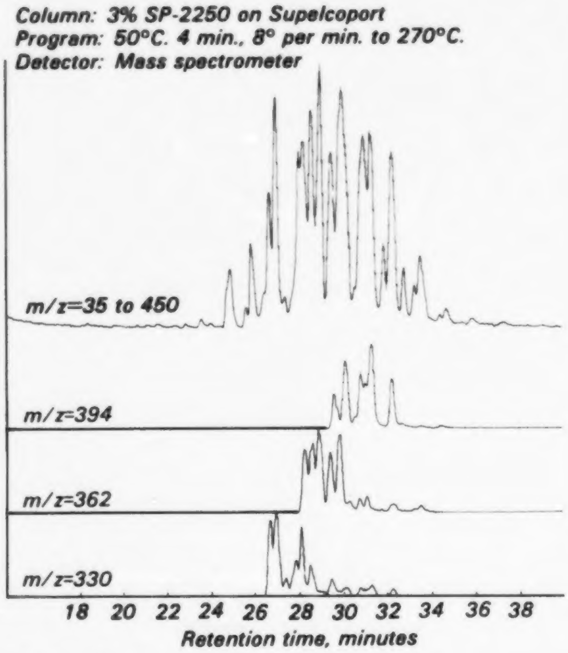
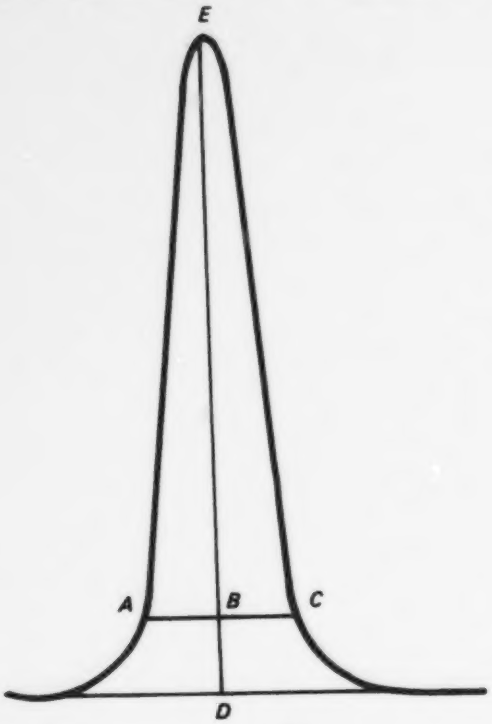


Figure 12. *Gs chromatogram of PCB-1260.*



Tailing Factor $= \frac{BC}{AB}$

Example calculation:

Peak Height = DE = 100mm
10% Peak Height = BD = 10 mm
Peak Width at 10% Peak Height = AC = 23 mm
AB = 11 mm
BC = 12 mm
Therefore: Tailing Factor $= \frac{12}{11} = 1.1$

Figure 13. *Tailing factor calculation.*

Appendix A

Definition and Procedure for the Determination of the Method Detection Limit

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero and determined from analysis of a sample in a given matrix containing analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicability to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

   (a) The concentration value that corresponds to an instrument signal/noise ratio in the range of 2.5 to 5. If the criteria for qualitative identification of the analyte is based upon pattern recognition techniques, the least abundant signal necessary to achieve identification must be considered in making the estimate.
   (b) The concentration value that corresponds to three times the standard deviation of replicate instrumental measurements for the analyte in reagent water.
   (c) The concentration value that corresponds to the region of the standard curve where there is a significant change in sensitivity at low analyte concentrations, i.e., a break in the slope of the standard curve.
   (d) The concentration value that corresponds to known instrumental limitations.

   It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent water (blank), prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated MDL. (Recommend between 1 and 5 times the estimated MDL.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated MDL, proceed to Step 4.

If the measured concentration of analyte is less than the estimated MDL, add a known amount of analyte to bring the concentration of analyte to between one and five times the MDL. In the case where an interference is coanalyzed with the analyte:

If the measured level of analyte is greater than five times the estimated MDL, there are two options:

(1) Obtain another sample of lower level of analyte in same matrix if possible.
(2) The sample may be used as is for determining the MDL if the analyte level does not exceed 10 times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the MDL and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If blank measurements are required to calculate the measured level of analyte, obtain separate blank measurements for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically deirable to evaluate the estimated MDL before proceeding with 4a. This will: (1) prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an incorrect MDL can be calculated from data obtained at many times the real MDL even though the background concentration of analyte is less than five times the calculated MDL. To insure that the estimate of the MDL is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a significantly lower MDL. Take two aliquots of the sample to be used to calculate the MDL and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in the desirable range for determining the MDL, take five additional aliquots and proceed. Use all seven measurements to calculate the MDL.
(2) If these measurements indicate the sample is not in the correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2 \Big/ n\right]$$

$$S = (S^2)^{1/2}$$

where: the x_i, i = 1 to n are the analytical results in the final method reporting units obtained from the n sample aliquots and $\sum_{i=1}^{n} X_i^2$ refers to the sum of the X values from i = 1 to n.

6. (a) Compute the MDL as follows:

$$MDL = t_{(n-1,\ 1-\alpha\ =\ .99)}(S)$$

where:

MDL = the method detection

$t_{(n-1,\ 1-\alpha\ =\ .99)}$ = the students' t value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. See Table.

S = standard deviation of the replicate analyses.

(b) The 95% confidence limits for the MDL derived in 6a are computed according to the following equations derived from percentiles of the chi square over degrees of freedom distribution (X^2/df) and calculated as follows:

MDL_{LCL} = 0.69 MDL
MDL_{UCL} = 1.92 MDL

where MDL_{LCL} and MDL_{UCL} are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimated MDL and calculated MDL of subsequent MDL determinations.

(a) If this is the initial attempt to compute MDL based on the estimated MDL in Step 1, take the MDL as calculated in Step 6, spike in the matrix at the calculated MDL and proceed through the procedure starting with Step 4.
(b) If the current MDL determination is an iteration of the MDL procedure for which the spiking level does not permit qualitative identification, report the MDL as that concentration between the current spike level and the previous spike level which allows qualitative identification.
(c) If the current MDL determination is an iteration of the MDL procedure and the spiking level allows qualitative identification, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F ratio.

$$\text{if} \quad \frac{S_A^2}{S_B^2} < 3.05$$

then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{1/2}$$

if $\frac{S_A^2}{S_B^2} > 3.05$, respike at the last calculated MDL and process the samples through the procedure starting with Step 4.
(c) Use the S_{pooled} as calculated in 7b to compute the final MDL according to the following equation:

$$MDL = 2.681\ (S_{pooled})$$

where 2.681 is equal to $t_{(12,\ 1-\alpha\ =\ .99)}$.

(d) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from percentiles of the chi squared over degrees of freedom distribution.

MDL_{LCL} = 0.72 MDL
MDL_{UCL} = 1.65 MDL

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

Reporting

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to

B180/9

determine the MDL must also be identified with the MDL value. Report the mean analyte level with the MDL. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, report the mean recovery, and indicate if the MDL determination was iterated.

If the level of the analyte in the sample matrix exceeds 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

Reference

Glaser, J. A., Foerst, D. L., McKee, G. D., Quave, S. A., and Budde, W. L., "Trace Analysis for Wastewaters," *Environmental Science and Technology, 15,* 1426 (1981).

Table of Students' t Values at the 99 Percent Confidence Level

Number of Replicates	*Degrees of Freedom (n-1)*	$t_{(n-1,\ 1-\alpha = .99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
∞	∞	2.326

• U.S. GOVERNMENT PRINTING OFFICE: 1982-559-092/0443